VENTRICULAR TACHYCARDIA AND DEATH: A STUDY OF DRUG PROTECTION AND POTENTIATION IN VARIOUS CANINE MODELS

by

Andrew Charles Geoffrey Uprichard

MB., ChB. (Edin) 1981

MRCP. (UK) 1984

A Thesis submitted to the

Faculty of Medicine

University of Edinburgh

In fulfillment of the requirements

for the degree of

Doctor of Medicine

1988

UNIVERSITY OF EDINBURGH

ABSTRACT OF THESIS (Regulation 7.9)

Name of Candidate UPRICHARD, Andrew Charles Geoffrey

Address .....

Degree M.D. *Date* December, 1987

Title of Thesis Ventricular Tachycardia and Death: A Study of Drug Protection and Potentiation Using Various Canine Models

No. of words in the main text of Thesis Approx 50,000.

The thesis involves the evaluation of a number of antiarrhythmic agents in various experimental canine models. Following the introduction, chapter 1 describes current concepts on cardiac electrophysiology (EP) and arrhythmia production; ischaemic heart disease, myocardial infarction and ventricular fibrillation are described, and the prognostic importance of chronic ventricular ectopy is discussed. Chapter 2 involves the management of ventricular arrhythmias and deals mainly with antiarrhythmic drugs and their classification. Current ideas on the role of drugs in the prophylaxis of sudden cardiac death are discussed. Chapter 3 concerns various animal models used for the study of antiarrhythmic drug action and includes non-canine, acute canine and chronic canine (including programmed electrical stimulation[PES]) sections. Chapter 4 describes the methods used for the studies and the statistical analyses employed.Chapters 5 and 6 involve the evaluation of a myocardial-selective α_1- adrenoceptor antagonist, UK-52046. In chapter 5, the drug is compared with atenolol in a number of automaticity models, including the ouabain-induced arrhythmia, halothane-adrenaline arrhythmia, the arrhythmia 24 hours after coronary artery ligation (CAL) and the adrenaline-induced arrhythmia after CAL. The same drug was studied in the reentrant arrhythmias of PES and acute coronary ischaemia in chapter 6. Chapter 7 describes the comparison of a class 1 antiarrhythmic agent (mexiletine) with a β adrenoceptor antagonist (atenolol) against the arrhythmias produced by PES, and correlates the results with a number of EP parameters. Chapter 8 is an antiarrhythmic and EP study involving a comparison of the racemic mixtures of tocainide and mexiletine with their respective stereospecific enantiomers. Chapter 9 describes a placebo-controlled study which investigated the arrhythmogenic actions of the H_2 receptor antagonists cimetidine and ranitidine and compared the results with quinidine. In chapter 10, an analysis is made of infarct sizes for all 119 animals prepared for PES. Reduction of infarct size was studied as a function of time after CAL and correlated with inducibility of arrhythmias on PES. A discussion of the results from chapters 5 to 10 in made in the final chapter, which also includes a critical overview of the work and some proposals for the future. Appendices 1 and 2 include the chemical structures of the drugs used and published material arising from the studies. The thesis ends with a bibliography.

INDEX

PREFACE

This thesis describes the evaluation of various experimental models used for the testing of a number of new and established antiarrhythmic drugs. All the work described herein was carried out in the department of Therapeutics and Pharmacology, Queens University, Belfast during the period 1985-1987, during which time the author was in receipt of a DHSS (N.Ireland) research fellowship.

AKNOWLEDGEMENTS

I should like to aknowledge my thanks to the following:

The Eastern Health and Social Services Board for Northern Ireland and the Queens University of Belfast for its award of a research fellowship during the period 1985-1987.

Dr. D.W.G. Harron for his supervision during the tenure of my fellowship, his sincere enthusiasm for the subject, and his encouragement throughout.

Professor R.G. Shanks for his advice, guidance and support during my time in his department.

Mr. R. Wilson for his invaluable technical help in all aspects of the work involved in this thesis.

The Staff of the Medical Research Unit for the professional assistance afforded for the surgical procedures.

My colleagues in the Department of Therapeutics.

And my family for their love and care.

DEDICATION

To Bryan David Uprichard, Flying Officer (RAF), killed 28th August, 1974 aged 23 years.

To Chris, Rebecca and Jaime, for all the love they have brought.

Introduction and Aims

From lightening and tempest; from plague,
pestilence and famine; from battle and murder,
and from sudden death, Good Lord, deliver us.

The Litany, Book of Common Prayer

Despite the innumerable technological and medical advances which have occurred since the writing of the Litany, it remains clear that sudden death is as real a problem today as it was in the 16th century. In medical terms, 'sudden death' generally denotes death which is nonviolent, which is unexpected, which is witnessed and which is instantaneous or occurs within a few minutes of an abrupt change in previous clinical state (Roberts, 1986). Since diseases of the heart are by far the most common cause for people to die suddenly, the word 'cardiac' is usually included, and the term 'sudden cardiac death' applied to persons who die suddenly from ischaemic heart disease (Eisenberg et al, 1986). An idea of the magnitude of the problem can be gauged from epidemiological data which suggest that sudden cardiac death may account for up to 30% of all deaths (Madsen, 1985). This is further compounded by the observation that in nearly 25% of these individuals, sudden cardiac death is the first manifestation of underlying heart disease (Kuller, 1966). The development of modern electrocardiographic monitoring however, has at least allowed us better understanding of the electrophysiological basis of the problem, and several reports have identified ventricular fibrillation as the most common terminal mechanism (Lown and

Graboys, 1977; Gradman et al, 1977; Hinkle et al, 1977; Nikolic et al, 1982). Furthermore, it appears that in the majority of cases ventricular fibrillation is a primary event and not secondary to acute myocardial infarction (Kuller et al, 1972; Baum et al, 1974; Lucchesi, 1984).

Since, once established, the only management of ventricular fibrillation is the application of a direct current counter-shock (Lown et al, 1962), for many years researchers and clinicians alike have looked for treatable factors that might be identified in those at risk of sudden cardiac death. While these people can be shown to share a high prevalence of risk factors for ischaemic heart disease, they cannot unfortunately be distinguished from other patients with ischaemic heart disease (Doyle et al, 1976). However, studies of patients after myocardial infarction have been able to identify a number of risk factors for subsequent sudden cardiac death, and of these, chronic ventricular ectopy appears to be of prime importance (The Coronary Drug Project Research Group, 1973; Moss et al, 1979; Hinkle, 1981; The Multicenter Postinfarction Research Group, 1983; Mukharji et al, 1984; Bigger et al, 1984). As a result, there is now an increasing awareness of the need for compounds with effective antiarrhythmic activity in the management of such patients.

The Lord hath created medicines out of
the earth; and he that is wise will not abhor them.
With such doth he heal men, and taketh away their pains.

Ecclesiasticus 38, 4-7.

The use of antiarrhythmic drugs may date back as far as 1749, when Jean-Baptiste de Sénac used cinchona for the treatment of 'rebellious palpitation' (Roden and Woosley, 1984). Although the synthesis of the dextro-isomer of quinine was carried out by Pasteur in the 19th century, it was not until 1914 that Wenchebach, the Viennese physician, employed the drug for the management of atrial fibrillation (Hoffman and Bigger, 1971). Since then, however, the number of antiarrhythmic drugs has grown enormously, and over the past 10 years a new compound has become available approximately every six months (Vaughan Williams, 1984). When one considers that, for every new drug presented, several more will have failed to reach full development, one can gain an idea of the magnitude of the field of antiarrhythmic therapy. At the same time, it becomes clear that such research and development would not be necessary if the ideal antiarrhythmic drug were available. Such a drug might be expected to possess certain favourable characteristics such as low cost, simple dosage, broad spectrum of action and freedom from side effects, but at the end of the day the ultimate attribute will be the ability to save lives. While many of the currently available antiarrhythmic drugs can significantly reduce the incidence of ventricular arrhythmias, recent reports have suggested that there is no associated improvement in mortality (Myerburg et al, 1979; May et al, 1983; Furberg et al, 1983; Impact Research Group, 1984). It is known that ß adrenoceptor blockade can reduce mortality after myocardial infarction

(Multicenter International Study, 1975; Norwegian Multicenter Study Group, 1981; Beta-Blocker Heart Attack Trial Research Group, 1982) but it has been suggested that this relates more to an anti-ischaemic than any antiarrhythmic action of the drugs (The Miami Trial Research Group, 1985; ISIS-1 Collaborative Group, 1986). There have even been a number of reports linking the use of antiarrhythmic drugs with a worsening of the arrhythmia being treated (Velebit et al, 1982; Ruskin et al, 1983; Poser et al, 1985; Torres et al, 1985).

Thus it is necessary that we continue the search for a safe, effective antiarrhythmic compound that will improve both the quality and the expectancy of life for the patient after myocardial infarction.

Let his heart be changed from
man's and let a beast's heart be
given unto him.

Daniel 4, 16

Ideally the best model for the careful study of antiarrhythmic action will be the patient with heart disease whose health is disturbed or whose life is at risk as a result of cardiac arrhythmias. Whilst this may be justifiable with drugs which have been shown to possess potentially life-saving characteristics, it will be wholly inappropriate for the initial screening investigations required of all antiarrhythmic agents. In such cases, it becomes necessary to substitute experimental models in the form of isolated tissue

preparations or intact animals. As our understanding of the many factors involved in the genesis of arrhythmias has developed, so too have these models progressed from the relatively simple, inexpensive methods previously employed to the highly sophisticated models which now attempt to take into account those pathophysiological variables (such as collateral circulation, neurohumeral influences and underlying arterial diseases) which pertain in the human situation.

The aim of this thesis has been to gain a better understanding of the various factors involved in the genesis of those malignant ventricular arrhythmias which are responsible for sudden death in man, and to study the effects (both beneficial and deleterious) of several pharmacological compounds on ventricular arrhythmias generated in a number of experimental animal models. Some of these models have been employed in similar studies for many years, while one (programmed electrical stimulation in the chronic myocardial infarction model) represents a recent addition to the armoury of the experimental pharmacologist. Each model is critically appraised with regard to its applicability to the human situation, and results with antiarrhythmic drugs are compared with previous studies, both experimental and in man.

Chapter 1. THE HEART

1.1 Electrophysiology of the Heart

Evolutionary development has done much for the series of tubular cardiac structures, still present in certain primative organisms, which are now apparent as the four-chambered, four valved, dual actioned organ of modern vertebrates (Davis, 1927). Nevertheless the fundamental action of the heart remains that of a pump, and as such depends upon regular, repetitive contractions which will carry the oxygen (as blood) across its length. These contractions are in turn controlled by a complex series of electrical events, mediated by the movement of charged ions across the membranes of the cardiac cells.

1.1.1. The Action Potential

Within a resting cardiac cell there is normally a negative potential of approximately 90mv relative to the extracellular space (the transmembrane potential). This potential is maintained by an energy-dependent membrane pump which actively extrudes sodium ions (Na^+) from within the cell. The same pump is also responsible for a net inward movement of potassium ions (K^+), resulting in an intracellular K^+ concentration of approximately 35 times that of the extracellular fluid, while the Na^+ concentration is approximately 5 times greater in the extracellular fluid. The transmembrane potential is thus maintained by an equilibrium where electric gradients (for example tending to move K^+ into the cells) are exactly balanced by concentration gradients (tending to extrude K^+ from

cells) and no net ionic movement occurs. Should the transmembrane potential be increased (i.e. moved towards zero) for any reason, a potential exists (the threshold potential) where the mechanisms described above temporarily break down and there is a reversal of ionic concentrations. Such events are termed the action potential; they are responsible for initiating myocardial contraction and are represented on the surface electrocardiogram as the QRS complex and T wave.

1.1.2. The Fast Response

Intracellular recordings of the same sequence of events appear rather different, and are represented in figure 1.1. Having reached the threshold potential, the initial rapid depolarization and overshoot are due to a rapid increase in Na^+ permeability (phase 0 of the action potential). This fast Na^+ channel (i_{Na}- where i is the representation for all membrane currents) may be viewed as being guarded by a 'gate', or charge-dependent configuration of the phospholipids within the cell membrane (Wallace, 1982). During phase 0, the membrane potential reaches approximately +20 mv, but never quite reaches the +50 mv predicted value since activation of the fast Na^+ channel lasts only 1-2 msec before becoming inactivated (phase 1) (McAllister et al, 1975).

In addition to the fast sodium channel, there is a second and much more slowly inactivated inward current which is responsible for the plateau phase of the action potential (phase 2). This 'slow'

current (i_{si}) is mediated by the flux of sarcolemmal calcium (Ca^{++}) which is essential for contraction. Originally thought to be just one channel, recent work has shown that i_{si} probably involves a number of separate mechanisms (Hart, 1985). Repolarization of the action potential (phase 3) is brought about by an outward K^+ movement (i_K) which activates slowly on depolarisation but deactivates rapidly once repolarisation has taken place (Hart, 1985). As the plateau phase of the action potential is responsible for the ST segment of the ECG, so repolarization corresponds with the T wave.

1.1.3. **Refractoriness**

During the sequence of events described, the myocardial cell will be unable to conduct another impulse; this so-called refractory period can be divided into an absolute refractory period, corresponding to the period from the time threshold is reached until the point where repolarization has returned the membrane potential to at least -50 mv (Weidmann, 1955) and a relative refractory period, lasting from this point to the return of the normal resting membrane potential. During the absolute refractory period no stimulus, no matter how strong, will produce an action potential, but during the relative refractory period stronger than normal stimuli can cause excitation. The effective refractory period (ERP) is the main determinant of the number of propagated impulses the heart can generate (Singh and Nadamanee, 1982); it is often used as an experimental parameter for measuring the shortest interval

between 2 stimuli capable of conducting respective beats (Simson et al, 1979) (figure 2.1). The functional refractory period (FRP) is an equivalent of the ERP but also inversely related to conductivity. (Ferrier and Dresel, 1974). Usually used as an index of refractoriness at the AV node, the FRP may, however, be employed for any area of myocardium under study.

1.1.4. The Slow Response

While the above description (the 'fast' response) is characteristic of normal atrial and ventricular muscle cells and of Purkinje fibres, the corresponding action potentials for the pacemaker cells of the sinus and atrioventricular nodes appear altogether different (figure 1.2). In these fibres, resting membrane potential is -40 to -70 mv (cf. -80 to -90 mv in 'fast' response fibres), upstroke velocity is very slow, resulting in the term 'slow' response fibres. The most striking difference between the two action potential configurations is the spontaneous diastolic depolarisation (phase 4) seen in the pacemaker cells. The ionic basis of this appears to lie in a decrease in the outward K^+ current (i_K- but known as i_{x1} in pacemaker tissue) allowing an inward current of Na^+ (i_f) to initiate depolarisation (Hart, 1985). Furthermore, since slow-response action potentials are not altered by tetrodotoxin (which blocks i_{Na}) but are abolished by manganese or verapamil (which antagonise i_{si}), it appears that they are mediated solely by

slow (Ca^{++}) currents (Wit and Cranefield, 1974).

Under physiological conditions, slow-response action potentials play an important role in the propagation of impulses within nodal tissues; this is particularly so at the atrioventricular node, where they ensure optimal cardiac performance by delaying ventricular systole until after atrial contraction (Zipes and Fischer, 1974). Yet the slow response is not recorded from these types of fibre only, as disease processes may alter the ionic mechanisms underlying action potential generation in non-conducting tissues such that they also show slow response activity. Thus diseases may convert fast atrial and ventricular fibres into slow-response fibres (Wit et al, 1974 (a)). The nature of the slow response (viz. low rate of depolarization and low amplitude of action potential) results in extremely slow conduction, and has been held responsible for the reentrant arrhythmias responsible for sudden death in man (Sperelakis, 1984).

1.1.5. **The Effects of Ischaemia**

Mention has already been made of the slowing of action potential propagation which may occur in ischaemic conditions (1.1.4.). Yet this is only one of a number of electrophysiological derangements, the understanding of which is gaining increasing importance as clinicians and electrophysiologists alike attempt to define the conditions contributing to life-threating arrhythmias in man. It is known, for example, that acute ischaemia causes

depolarisation of the resting membrane, a decrease in the rate of rise and overshoot of the action potential and a decrease in action potential duration (Prinzmetal et al, 1961; Russell et al, 1979). Conduction initially speeds, before slowing to the point of fractionation or block (Boineau and Cox, 1973; Williams et al, 1974) and similar changes are apparent with refractory periods (Russell and Oliver, 1978). These abnormalities result from the metabolic, ionic and neurohumeral changes which result from the abrupt interruption of coronary blood flow (Gettes, 1986); while many of these changes have yet to be identified, an idea of the arrhythmogenic nature of ischaemia may be gained by looking at the electrophysiological effects of the individual components of ischaemia.

The dominant effect of hypoxia is to shorten the action potential duration in all cardiac cell types (Morena et al, 1980). This is thought to be mediated by increased potassium efflux from the cell (Vleugels and Carmeliet, 1976). Since shortening of action potential duration is likely to be paralleled by similar changes in refractoriness, this represents an inherently arrhythmogenic process, since shortening of refractionness may allow early depolarization of fibres in an adjacent reentrant pathway (1.2.3.).

An increase in extracellular potassium results in a number of electrophysiological derangements, including depolarization of the membrane potential and reduction in the rate of rise of the action potential upstroke (phase 0), lowering the action potential

amplitude and plateau potential (phase 2), accelerating rapid repolarization (phase 3), and decreasing the spontaneous diastolic depolarization (phase 4) (Weidman, 1956; Hoffman and Cranefield, 1960). The initial effect of this is to render the myocardium more excitable; although further increases in potassium decrease excitability, conduction then becomes impaired, so maintaining the arrhythmogenic environment (Gettes, 1986).

The major electrophysiological effect of acidosis is a slowing of conduction (Gettes, 1986). This may be associated with a decrease in the rate of rise of the action potential, but it is not thought to be a cause-and-effect phenomenon; rather it is believed that the slowing of conduction relates to alterations in cellular uncoupling (Gettes, 1986). Acidosis has also been shown to decrease influx and efflux of calcium, and this has been reported as a mechanism for the induction of slow response activity (Davis et al, 1976).

Under physiological conditions, the predominant adrenergic receptor in the heart is of the ß type, where it mediates increased spontaneous depolarizations in both fast- and slow-response fibres, enhances speed of conduction, shortens refractory periods and increases the force of contraction (Sharma and Corr, 1983). These effects have been attributed to catecholamine-induced increases in cyclic AMP (Tsien, 1972). Under ischaemic conditions, there are known to be increases in both circulating (Richardson, 1963) and neurogenic (Corr and Gillis, 1978) catecholamines, and enhanced

ß-stimulation may be arrhythmogenic by increasing oxygen demand, contributing to hypokalaemia (Struthers et al, 1981) and favouring the emergence of slow-response activity (Sharma and Corr, 1983). It has also been suggested that enhanced α adrenergic responsiveness may be a feature of ischaemia (Sheridan et al, 1980) and further contribute to arrhythmia production by its effects on intracellular calcium fluxes (Corr and Sharma, 1984). These points will be discussed in more detail at a later stage (chapters 5,6,11.).

1.2. The Arrhythmia Concept

The term arrhythmia* can be applied to any condition which affects the normal sequence of an impulse arising in the sinoatrial node and resulting in a normal atrial activation coupled at a physiological interval to a normal ventricular contraction. By definition therefore, we should include conditions of atrioventricular block, bundle branch and fascicular blocks and bradyarrhythmias as well as the various tachyarrhythmias which more commonly spring to mind. However, having described the electrophysiological bases for arrhythmia genesis, the discussion

** Strictly speaking the term arrhythmia applies (as do all conditions with the prefix a-, or an-) to a condition where no rhythm exists, i.e. asystole. Semantics would therefore dictate we use the word dysrhythmia. Unfortunately, however, old habits die hard and I use the former term with these shortcomings accepted.*

will therefore be confined to the ventricular tachycardias (premature ventricular contractions, paroxysmal and sustained ventricular tachycardia, and ventricular fibrillation) which are the cause of such great concern in our society today.

It has become customary to consider the mechanism thought to be responsible for the genesis and perpetuation of cardiac arrhythmia under three headings, viz. disorders of impulse formation, disorders of impulse conduction, and a combination of both of these mechanisms

1.2.1. Automaticity

Reference has already been made to the ability of cells within the conducting system of the heart to undergo spontaneous depolarization. This property is termed automaticity, and may be seen also in certain specialized atrial fibres, in fibres around the coronary sinus and mitral and tricuspid valve leaflets, and in the His-Purkinje system (Hoffman and Cranefield, 1960). Automaticity is more common is slow-response fibres, where it is thought to depend on a balance between i_{x1} and i_f (Hart, 1985) but it is likely that a number of ionic mechanisms may initiate spontaneous depolarisation, since:

(a) alterations in ionic environment may affect the automaticity of different fibres in different ways.

(b) drugs may abolish automaticity in some fibres without affecting others.

(c) automatic fibres respond in different ways to electrical stimulation (Wit et al, 1974 (a)).

Fast fibres (particularly those of the specialized conducting system) are also capable of automaticity and it appears that the resultant action potentials may be of the slow or fast varieties (Wit et al, 1974 (a)).

The pacemaker site of the heart is that site (usually the SA node) with the great degree of automaticity. However, if this is suppressed, or if conduction of the impulse is impaired (for example by vagal stimulation), then the pacemaker function may be taken over by 'lower' sites within the conducting system. Similarly, enhancement of automaticity (for example by disease processes) may result in a shift of the site of the pacemaker to an ectopic focus (Vassalle, 1971), where initiation of an impulse may spread to involve all of the heart, resulting in atrial or ventricular tachyarrhythmias. An example of this can be seen in the increased spontaneous diastolic depolarization of Purkinje fibres surviving experimental coronary artery occlusion in dogs (Friedman et al, 1973).

If an automatic focus is interrupted by electrical stimulation, providing the rate of stimulation exceeds the automaticity of the arrhythmic focus, the stimuli will 'capture' the heart beat and override the arrhythmia ('override suppression'). Cessation of stimulation will then be followed by a period of quiescence before the ectopic rhythm gradually re-establishes itself (Vassalle, 1970).

Such a response to stimulation is generally taken as implying the automatic nature of an arrhythmia.

1.2.2. Triggering

One feature which distinguishes automaticity from disorders of impulse propagation is the ability to arise spontaneously and independent of any initiating impulse. Under certain circumstances, however, automatic foci may require such activation, and will remain quiescent in the absence of such a 'trigger' (Hoffman and Rosen, 1981). These are triggered arrhythmias, and have been further categorised into those initiated by early afterdepolarizations and late afterdepolarizations (Cranefield, 1977).

Arrhythmias due to early afterdepolarizations occur when the fibre fails to repolarize completely after generating an action potential. As the membrane potential lingers at intermediate values, oscillatory depolarizations can arise, attain threshold and initiate further responses (Hoffman and Rosen, 1981). Delayed afterdepolarizations occur after phase 3 of the action potential has restored the negative membrane potential, but usually to a value somewhat less (negative) than normal (Hoffman and Rosen, 1981).

Since many triggered arrhythmias can be reduced or abolished by agents which antagonize the slow channel (Rosen et al, 1974), it might appear that such arrhythmias result from activation of slow response fibres; However, afterdepolarizations have also been identified in fast response units (Cranefield, 1980).

1.2.3. Reentry

Under physiological conditions the cardiac impulse, having sequentially activated the atria and ventricles, will terminate spontaneously since it will be surrounded by recently excited and thus refractory tissue. The concept of reentry (first described by Mines in 1913) implies that the impulse persists within the myocardium to re-excite areas no longer refractory and thus set up a continuous circuit of impulse generation, the so-called 'circus' movement. A functional 'loop' of myocardium is not a difficult concept, since many cardiac fibres can conduct in 2 directions and cross-connections between fibres have been demonstrated (Gallagher, 1982). However, a reentrant circuit does depend on there being an area of unidirectional block within the myocardium, and also that the speed of the impulse is sufficiently slow to allow recovery of the proximal fibres before re-excitation by the wave-front (Wit et al, 1974 (b)).

In order for an impulse to travel retrogradely along a reentrant pathway requires the presence of an area which is unable to conduct the uniform antegrade depolarization of an approaching impulse; if the proximal tissues have by this stage recovered from refractionness, the conditions for reentry are established (figure 1.3).

Considering the rapid velocity of conduction of normal atrial and ventricular cells, it was previously thought that reentry would

be impossible outwith the AV node, as the loop would have to be unrealistically long to allow recovery of the proximal tissues (Wit et al, 1974 (b)). With the demonstration that disease states could alter the conduction velocity of fast fibres, however (Wit et al, 1974 (a)), came the realization that small reentrant pathways could be responsible for ventricular arrhythmias both in animals (El-Sherif et al, 1977 (a)) and man (Josephson et al, 1978 (a)).

While Ca^{++}-dependent, slow response fibres are almost certainly involved in the genesis of AV nodal tachycardias (Wit et al, 1974 (c)), the situation is less clear with ventricular arrhythmias. Mention has already been made regarding the conversion of fast to slow fibres in certain disease states, but whether these are true Ca^{++}-dependent slow fibres, or merely depressed fast fibres remains unclear (Sperelakis, 1984). Ca^{++} channel antagonists have been shown to possess ventricular antiarrhythmic activity in experimental models, but not in man (Opie et al, 1984).

Reentry has been further divided into 'ordered reentry' and 'random reentry' (Hoffman and Rosen, 1981). In the former, the arrhythmia occurs over a circumscribed path with a finite anatomical reality, such as AV nodal reentry. Fibrillation (whether atrial or ventricular), would be an example of random reentry, with a number of randomly changing pathways, varying with time and impulse propagation. The main advantage of this system appears to be the differentiation of the 'benign' (largely supraventricular) reentrant pathways from the potentially life-threatening

ventricular arrhythmias in man which have also been shown to depend on reentrant mechanisms (El-Sherif et al, 1977 (a); Josephson et al, 1978 (a), (b)).

Predictable and reproducible initiation and termination of a tachyarrhythmia by appropriately timed electrical stimulation has been considered the *sine qua non* of a reentrant mechanism (Wellens, 1977; Josephson et al, 1978 (b)). While this is generally the case, and contrasts with the overdrive suppression of automatic arrhythmias, it should be remembered that an appropriately timed stimulus may act as a trigger for the initiation of early or late afterdepolarizations (Wit et al, 1974 (a)) (1.2.2).

1.2.4. **Parasystole**

Having considered disorders of impulse formation (automaticity and triggering) and disorders of conduction (reentry), it is appropriate to consider also those arrhythmias arising from a combination of both mechanisms

Parasystole is an independent, ectopic rhythm which functions autonymously with the primary heart rhythm. Its pacemaker is protected from excitation from outside impulses by unidirectional block, helping it effectively to function as a fixed rate artificial pacemaker (Gallagher, 1982). Parasystole is more frequently encountered in chronically diseased hearts (Chung, 1968) but because the inherent rate of the parasystolic focus is usually slow (20-60 beats per minute), the phenomenon in itself is unlikely to be

life-threatening (Gallagher, 1982).

Arrhythmias may be a feature of any disease process directly affecting the heart. Similarly they may be the cardiac reflection of generalized metabolic, endocrine or infective processes. This accepted, however, there is no doubt that the commonest pathological process responsible for life-threatening arrhythmias in man is atheromatous narrowing of the coronary arteries-ischaemic heart disease.

1.3. Ischaemic Heart Disease

Relatively uncommon under the age of 40, the incidence of ischaemic heart disease (IHD) increases steadily with age. Under the age of 45, the incidence in men is more than 10 times that in women, but this difference becomes less apparent with increasing age so that in the over-65 age group the respective incidences are approximately equal (Julian, 1979).

While myocardial ischaemia may result from a number of processes, the term IHD generally applies to ischaemia of atheromatous origin. This is still not fully understood, but is thought to involve the deposition of fatty substances, especially cholesterol, in the arterial intima. Various risk factors have been identified for the process, and include hypertension, hyperlipidaemia, diabetes, cigarette smoking, family history, the degree of physical activity and possibly mental stress (Julian, 1979). Although this appears to be a process which starts early in

life (Zelis and Wenger, 1982) IHD nevertheless remains a clinical diagnosis, usually presenting as angina pectoris, acute myocardial infarction or sudden cardiac death.

1.3.1. Angina Pectoris and Acute Myocardial Infarction

Angina pectoris is a syndrome resulting from an imbalance between the oxygen demand of, and the oxygen supply to, the myocardium (Julian, 1979). It classically presents as a retrosternal pain, usually described as a 'tightness' or 'heaviness' which often radiates to the neck, jaw and arms. The pain is usually provoked by exercise, particularly after a heavy meal or in cold weather. Emotional upset may also act as a trigger, while some patients only experience attacks at night or when lying flat (angina decubitus). Cessation of exercise will usually terminate the attack, which characteristically lasts from 1 to 3 minutes. Pain lasting more than 3 minutes should raise the suspicion of myocardial infarction (see below).

Most patients with angina pectoris manage to live a fairly normal life, although they may have to modify their daily routine to avoid precipitating attacks. The danger to the patient is not of the angina attack itself, but of the increased risk of going on to develop an acute myocardial infarction.

The essential pathological feature of acute myocardial infarction (a 'heart attack') is myocardial necrosis, usually the consequence of total occlusion of a coronary artery (Julian, 1979). It

often presents with features indistinguishable from a severe angina attack, but usually unrelated to exercise and tending to last more than 30 minutes. Treatment with anti-anginal vasodilators will be ineffective. The diagnosis can subsequently be confirmed by demonstrating the characteristic ECG changes (ST elevation, T wave inversion and development of Q waves) and identifying circulating enzyme markers (especially myocardial-specific creatine phosphokinase: CK-MB) from the necrotic area of myocardium. The complications of acute myocardial infarction can generally be divided into those occurring within the first 2-3 days (the early complications) and those which threaten the patient after this period (late complications).

Arrhythmias are by far the most important early complication of acute myocardial infarction, occuring in over 90% of patients within 4 hours (Pantridge et al, 1975). The indications for treating arrhythmias are twofold: firstly, to correct an arrhythmia (such as a marked bradycardia, heart block or tachyarrhythmia) which impairs cardiac output. These are particularly common in the acute phase, where they are often associated with autonomic disturbances (Pantridge et al, 1975). Secondly, there is the question of treating the so-called 'warning' arrhythmias that are thought to be harbingers of ventricular fibrillation. In the past it was considered that frequent, multifocal or the so-called 'R-on-T' ventricular ectopics were associated with a high risk of ventricular fibrillation and required immediate antiarrhythmic therapy (Mounsey, 1967;

Thomas et al, 1968). However, subsequent findings have suggested that this may be incorrect; ventricular arrhythmias may occur without preceding events, and 'warning arrhythmias' are as common in those who do not proceed to ventricular fibrillation as in those who do (Julian, 1986). Nevertheless, the occurrence of an early ventricular ectopic of the 'R on T' variety is often the trigger for ventricular fibrillation (figure 1.4).

The late complications of myocardial infarction include thromboembolic phenomena, the risk of reinfarction and/or sudden death, heart failure, ventricular aneurysm and a number of psychological and social difficulties the patient may have to face.

1.3.2. Sudden Cardiac Death

First described as a distinct entity almost a century ago (MacWilliam, 1889), the incidence of sudden cardiac death has been estimated at 2-3% per year in patients with ischaemic heart disease. The syndrome has been defined variously as death occurring within 30 seconds of the onset of acute symptoms ('instantaneous death') (Friedman et al, 1973) to that occurring within 2 hours (Helmers et al, 1976), 2 days (Paul and Schatz, 1971) or 6 days (Meade et al, 1968). A more acceptable definition now appears to be death which occurs within one hour of the onset of acute symptoms (Gordon and Kannel, 1971; Myerburg, 1978; Goldstein, 1982), although it should be remembered that symptoms may be entirely absent in some cases.

Although sudden death may occur in the setting of acute myocardial infarction, the majority of cases are not associated with myocardial necrosis (Kuller, 1972; Baum et al, 1974; Lucchesi, 1984). In most cases, death is the result of a primary electrical event within an unstable myocardial environment (Podrid, 1985) and usually occurs outwith the hospital environment (Spiekerman et al, 1962). Several studies with ambulatory electrocardiographic monitoring have identified ventricular fibrillation as the most common terminal mechanism (Gradman et al, 1977; Hinkle et al, 1977; Nikolic et al, 1982), often preceded by a variable period of unimorphic ventricular tachycardia (Panidis and Morganroth, 1983; Pratt et al, 1983; Kempf and Josephson,1984; Milner et al, 1985). Although an unstable electric milieu is a necessary prerequisite for ventricular fibrillation, it is not sufficient per se to cause sudden death. Recent evidence suggests that transient, acute ischaemia (whether produced by platelet aggregation, coronary artery spasm or autonomic activity) may well be the important triggering event which predisposes for the development of lethal arrhythmias, both in experimental models and in post-infarction patients (Gradman et al, 1977; Theroux et al, 1979; Podrid, 1985; Lynch and Lucchesi, 1987).

The fact that sudden cardiac death may strike suddenly and unexpectedly in the months following myocardial infarction has prompted much research into defining those post-infarction risk factors which might be modified in the hope of improving prognosis.

In general, two groups of risk factors have been identified: those indicating left ventricular dysfunction and those associated with arrhythmias.

A number of investigators have demonstrated an independent association between left ventricular failure and the risk of sudden death in post-infarction patients (the Multicenter Postinfarction Research Group, 1983; Bigger et al, 1984; Mukharji et al, 1984). While these studies have generally based their data on radionuclide ejection fractions, in general simple clinical observations appear to offer as valuable prognostic information as do more expensive or invasive ones (Julian, 1981).

It has been shown that ventricular arrhythmias noted during the early phase of acute myocardial infarction appear to have little influence upon the subsequent course (Vismara et al, 1975). The continued presence of such ectopy, however, has been identified as a risk for subsequent sudden cardiac death in numerous studies (Coronary Drug Project Research Group, 1973; Hinkle et al, 1977; Moss et al, 1979; Multicenter Postinfarction Research Group, 1983; Bigger et al, 1984; Mukharji et al, 1984). It is for this reason that the management of chronic ventricular ectopy has posed a serious problem to those involved with the care of post-infarction patients.

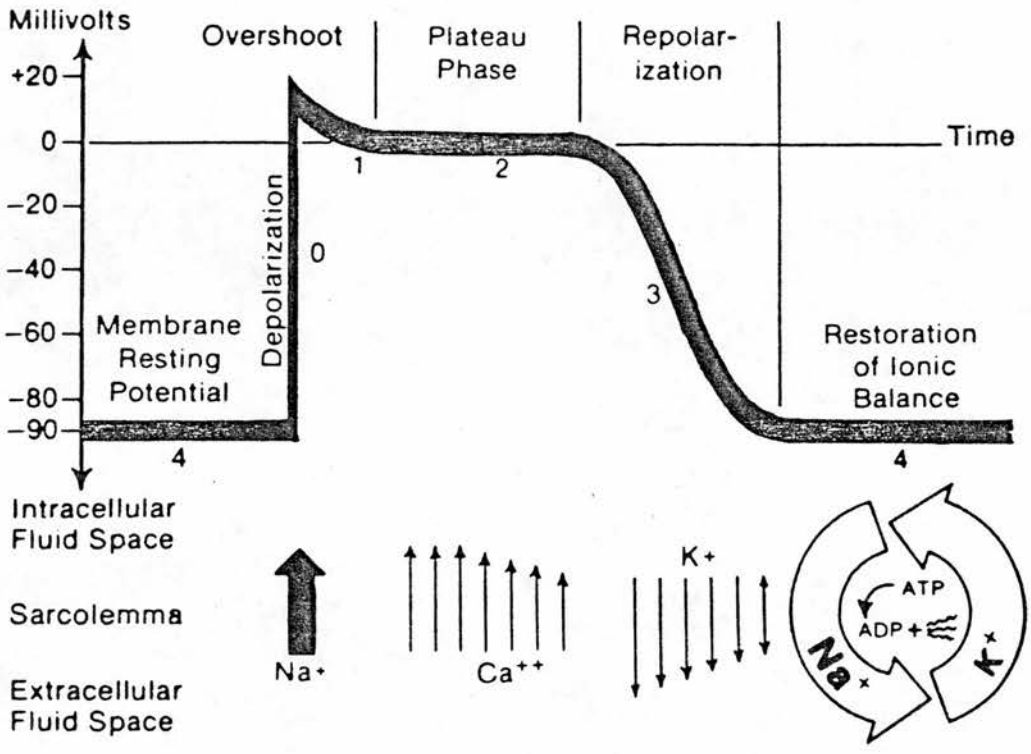


Figure 1.

Phases of the action potential of a cardiac muscle fibre and the corresponding changes in ionic conductance.

0, Depolarization.

1, Rapid Depolarization.

2, Plateau Phase.

3, Late Repolarization.

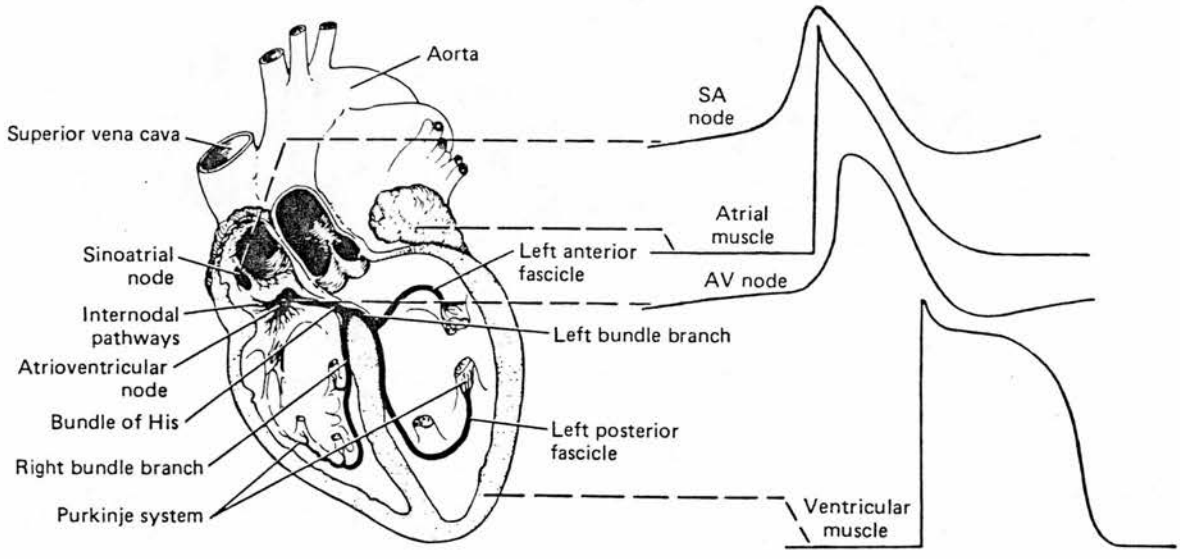


Figure 2.

Conducting system of the heart with typical transmembrane action potentials for the SA node, AV node, and atrial and ventricular muscle.

(Reproduced from: Ganong WF, ed. Review of medical physiology. California: Lange medical publications,1985.)

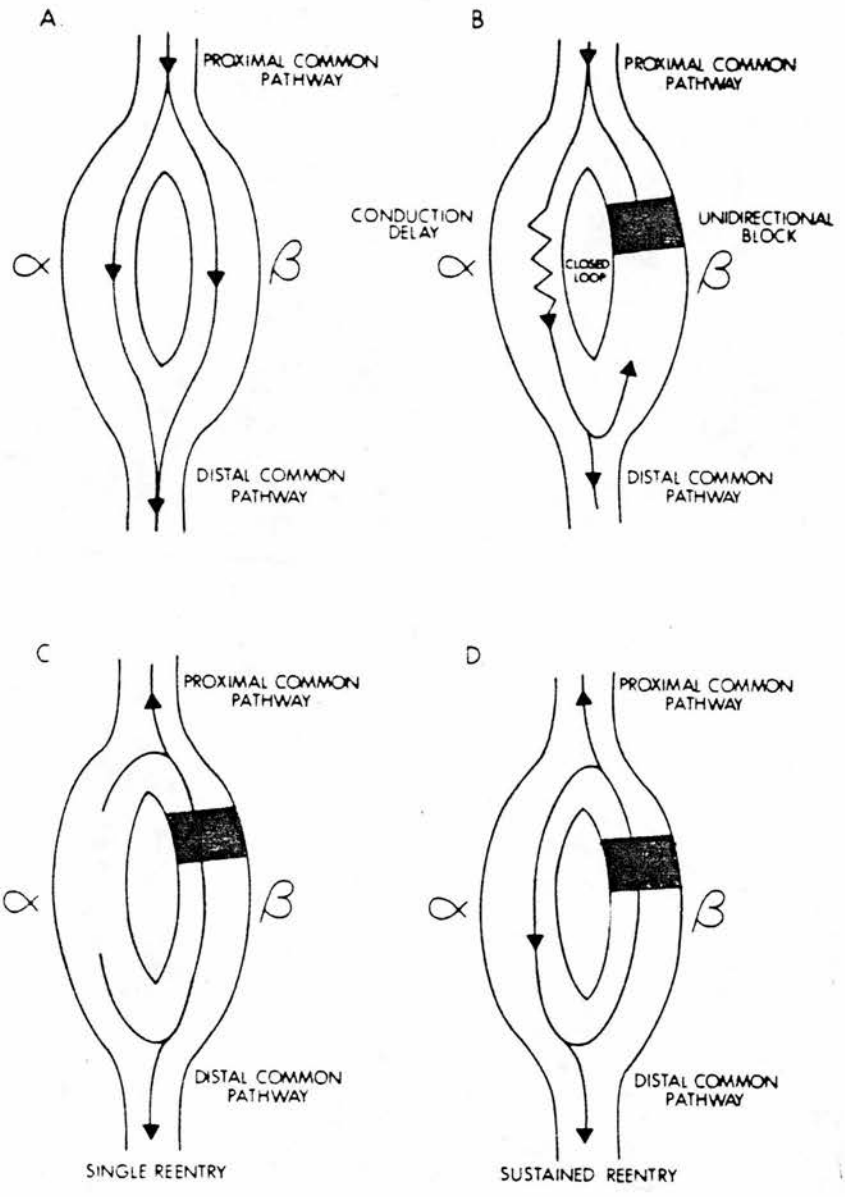


Figure 3.

Mechanism of reentry. The hypothetical circuit shows a proximal wavefront dividing into 2 divergent pathways (α,β), and uniting to form a distal common pathway.

A, Normal conduction in α and β.

B, Unidirectional block in β.

C, Impulse travels retrogradely along β.

D, Fibres in α sufficiently recovered to sustain reentry.

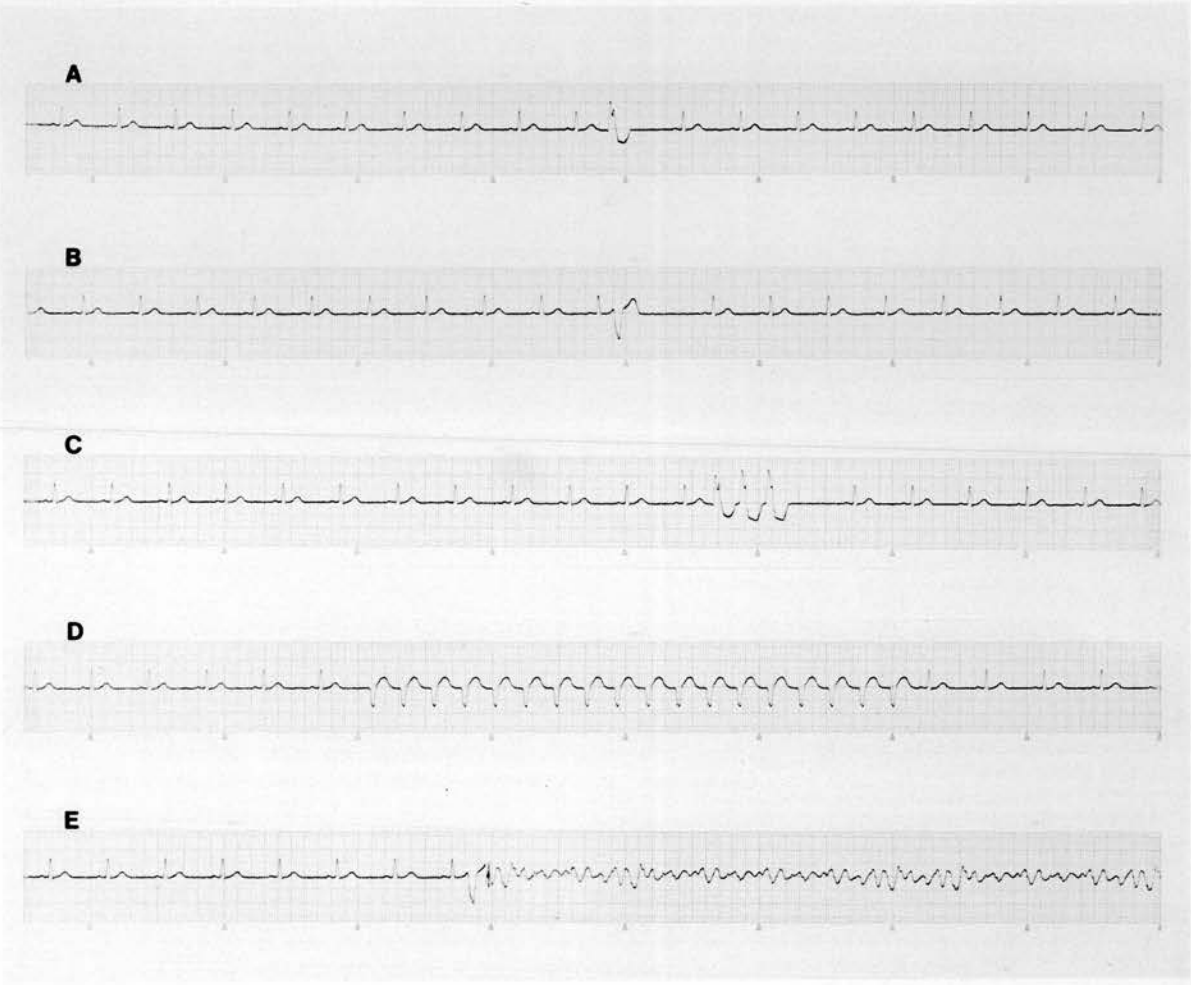


Figure 4.

Examples of arrhythmias:

A, Ventricular ectopic occurring late-cycle.

B, Early-cycle ('R on T') ventricular ectopic.

C, Salvo of 3 ventricular ectopics.

D, Short run of slow ventricular tachycardia.

E, ventricular fibrillation precipitated by R on T ectopic.

Chapter 2. MANAGEMENT OF CARDIAC ARRHYTHMIAS

2.1 Non-Drug Treatment

Whilst this thesis is largely concerned with the drug management of life-threatening ventricular arrhythmias, it is important to realize that other, non-pharmacological, methods exist for the same purpose. Accordingly, it is appropriate to discuss these briefly.

2.1.1 Implantable Defibrillators.

The reality of reversing ventricular fibrillation goes back to 1899, when Prevost and Battelli observed that a strong electric current could terminate experimental ventricular fibrillation (Prevost and Battelli, 1899). The first successful resuscitation of a patient appears to have been by Beck (1947), but the need for thoracotomy and direct application of the electrodes had obvious clinical limitations. Experimental closed chest defibrillation was first described in 1936 (Ferris et al, 1936) before being successfully demonstrated in man two decades later (Zoll et al, 1956). Lown et al (1962) demonstrated that direct current (DC) shocks were preferable to alternating current (AC) in terms of successful resuscitation, damage to the heart and paradoxical induction of arrhythmias. Such advances have led to the development of the mobile coronary care units, where resuscitating equipment can be brought to the patient at the first signs of myocardial infarction (Pantridge et al, 1975).

The introduction of an automatic implantable defibrillator in the late 1970's provided permanently available defibrillating capabilities to the patient at high risk of sudden death, without requiring specialized personnel or additional equipment (Mirowski et al, 1981). The device is programmed continuously to monitor the cardiac rhythm, to recognise ventricular tachycardia and ventricular fibrillation, and to deliver corrective defibrillatory charges when indicated (Mirowski et al, 1981). However, as the authors themselves recognise, "many problems remain to be solved and the ultimate value of this new therapeutic modality has yet to be determined".

2.1.2 **Automatic Pacemakers**

The first report of cardiac pacing for the management of ventricular tachyarrhythmias appears to have been in 1964 (Sowton et al, 1964). Since then, techniques have been modified and improved to the point where pacing now plays a major role in the diagnosis and management of arrhythmias (Griffin et al, 1981). McCallister et al (1966) described an implantable pacemaker capable of continuous rapid overdrive suppression of ventricular arrhythmias, before it was discovered that the same end could be achieved by bursts of rapid stimuli (Bennett and Pentecost, 1971). In 1978 Peters and co-workers reported on an externally controlled, radiofrequency pacemaker (Peters et al, 1978), and more recently we have seen the development of a multiprogrammable, automatically activating

ventricular burst pacemaker (Griffin et al, 1981). The principal hazards of these newer devices is acceleration of the tachycardia or induction of ventricular fibrillation, and they should therefore be instituted only after thorough electrophysiological assessment (Griffin et al, 1981).

2.1.3 Surgical Resection

It has been known for some time that patients with ventricular aneurysms exhibit a high frequency of ventricular arrhythmias (Sami et al, 1978). Following the reported benefit of surgical aneurysmectomy (Couch, 1959) this became a standard procedure, although long-term results of 'blind' aneurysmectomy have been equivocal (Sami et al, 1978; Gallagher, 1978). Recent interest has been renewed, however, with the technique of combining surgery with per-operative electrophysiological studies: using composite electrodes it is now possible during surgery to record electrical activity from various epicardial sites both in sinus rhythm and (induced) ventricular tachycardia ; from the resulting epicardial 'map' the cardiologist can then identify the point of origin of the arrhythmias and guide ventriculotomy accordingly (Fontaine et al, 1979; Horowitz et al, 1980; Mason et al, 1982).

2.2 Drug Treatment

2.2.1 Classification of Antiarrhythmic Drugs

From the time it was first recognised that drugs had an important role in the management of patients with heart disease, it was realised that the agents employed did not form an homogeneous group. They differed greatly in chemical structure, in pharmacokinetic characteristics and in their electrophysiological effects. This was particularly so with respect to the intracellularly recorded action potentials from different parts of the heart . For this reason it became desirable to identify those electrophysiological features which could be correlated with a particular therapeutic response; such an approach has formed the basis for the categorisation of actions of antiarrhythmic drugs (Vaughan Williams, 1970; Hoffman and Bigger, 1971; Singh and Vaughan Williams, 1972 (a); Vaughan Williams, 1975; Gettes, 1979; Vaughan Williams, 1984).

The classification which appears to have gained the greatest acceptance is that originally described by Vaughan Williams (1970). This is based on the effects of any antiarrhythmic compound being related to:

(i) depression of the fast response

(ii) inhibition of sympathethic activity to the heart

(iii) homogeneous prologation of the action potential duration

(iv) depression of the slow response.

.

Class 1	Class 2	Class 3	Class 4
Quinidine	Anti-Sympathetic	Amiodarone	Verapamil
Procainamide	Drugs	Bretylium	
Lignocaine		Sotalol	
Phenytoin			

Table 2.1

The original classification of antiarrhythmic drugs

(Vaughan Williams, 1975)

2.2.2 **Class 1**

These compounds (table 2.1) all share the property of being able to reduce the maximum rate of depolarization (MRD) in cardiac muscle. This they achieve by reducing the fast sodium current, which can be measured as a change in phase 0 of the action potential. Additional features of these drugs include an increase in the threshold of excitability, a depression in conduction velocity and a prolongation of the effective refractory period (ERP) (Singh and Nadamanee, 1982). Of these, prolongation of the ERP may be a particularly important process in explaining the actions of these

drugs in terminating reentrant pathways; this is most likely due to attachment of the drugs to sodium channels in their inactivated (ie. post-depolarization) state and prolonging that state for a variable period (Vaughan Williams, 1984).

More recently it has been suggested that class 1 should be further divided into three sub-groups (Harrison, 1983; Vaughan Williams, 1984) (table 2.2). This relates primarily to the effects on action potential duration (APD) by the drugs in the various groups. Thus class 1a drugs such as quinidine, procainamide and disopyramide prolong APD, class 1b drugs such as lignocaine, mexiletine and tocainide shorten APD, while class 1c drugs (encaidine, flecainide, lorcainide) have little effect. The pharmacological basis for this sub-grouping may well relate to the speed of dissociation of the drugs from the inactivated sodium channels (Vaughan Williams, 1984). Since shortening of APD is an inherently arrhythmogenic process (chapter 1.1.5), it is important to realize that class 1b drugs remain antiarrhythmic by nature of a relative increase in the ratio of effective refractory period to action potential duration (ERP:ADP ratio) (Harrison,1983) (figure 2.1).

The ability of all class I drugs to slow the maximum rate of depolarization (MRD) is often referred to as 'membrane stabilizing activity'. Similarly, since at 10 to 100 their antiarrhythmic concentration they behave as local anaesthetics in nerves, the

Class 1	a	b	c
Drugs	quinidine procainamide disopyramide	lignocaine mexiletine tocainide	lorcainide encainide flecainide
APD	prolonged	shortened	little change
ERP	lengthened absolutely and relative to APD	lengthened relative to ADP	little change

Table 2.2

Subdivisions of class I antiarrhythmic drugs

(adapted from: Vaughan Williams, 1984)

term 'local anaesthetic action' is frequently substituted to describe the drugs' action on the heart. As quinidine was the prototype of the group, the expression 'quinidine-like' is a further alternative.

2.2.3. **Class 2**

Class 2 antiarrhythmic drugs block sympathethic drive to the heart, and it should be noted that in Vaughan Williams' original

classification no restriction were placed as to where (anatomically or pharmacologically) this antagonism should occur (Vaughan Williams, 1975). Nevertheless, since sympathethic drive is mediated primarily by ß receptors, class 2 activity has largely become synonymous with ß-adrenergic blockade (Morganroth, 1985).

The ß-adrenergic antagonists (ß blockers) may be variously classified according to their respective hydro-or lipophilicity, their selectivity for the $ß_1$ receptor subtype or their intrinsic sympathomimetic (partial agonist) activity, but the common action on the heart appears to be depression of phase 4 depolarization (Singh and Nadamanee, 1982). For this reason the drugs are particularly useful in the management of arrhythmias dependent upon impulse transmission through the AV node (Singh and Nadamanee, 1982). Ventricular arrhythmias resulting from mitral valve prolapse, hypertrophic cardiomyopathy or in the setting of myocardial sensitization by anaesthetic agents may also respond to ß-adrenergic antagonists, but generally these drugs are only weakly antiarrhythmic in ventricular tachycardias (Morganroth, 1985). One exception may be where such arrhythmias are dependent upon enhanced sympathetic drive (chapter 1.1.5).

2.2.4 **Class 3**

One interesting aspect of the Vaughan Williams classification is that the third class of antiarrhythmic action existed as a theoretical proposition before a drug was found which met the

Drug	Potency (propanolol=1)	Cardio-selectivity	Partial Agonist Activity	Class 1 activity	N-octanolol:water Distribution Ratio
Acebutolol	0.3	+	+	+	0.68
Alprenolol	0.3	-	+	+	3.1
Atenolol	1	+	-	-	<0.02
Metoprolol	1	+	-	+	0.2
Nadolol	1	-	-	-	0.02
Oxprenolol	0.5-1	-	+	+	0.7
Pindolol	6	-	++	+	0.2
Practolol	0.3	+	+	-	<0.02
Propanolol	1	-	-	+	4.3
Sotalol	0.3	-	-	-	<0.02
Timolol	6	-	-	-	0.3

Table 2.3

Properties of the currently available β-adrenergic antagonists

required characteristics of the group. This was based on the

observation that in hyperthyroidism (where shortening of action potential duration is characteristic) arrhythmias are common, whereas in myxoedema (where action potentials are uniformly prolonged) arrhythmias are rare (Vaughan Williams, 1970). When the anti-anginal agent amiodarone was found uniformly to prolong action potential duration in association with significant antiarrhythmic activity for which no other explanation was apparent (Singh and Vaughan Williams, 1970), it became the prototype of the class 3 agents. It should be noted that the mechanism whereby amiodarone lengthens action potential duration and effective refractory period differs fundamently from the class 1a drugs. The latter produce a time-dependent increase in refractoriness by altering sodium conductance during depolarization whereas the class 3 drugs exert their effects via a voltage-dependent change in refractoriness by slowing repolarization (Singh and Nadamanee, 1982).

It is also important here to distinguish between homogenous prolongation of the action potential as occurs with class 3 drugs and is antiarrhythmic, and the heterogenous prolongation seen in the so-called 'long QT syndromes' (whether hereditary or acquired) which lead to spatial variation in refractoriness and subsequent arrhythmogenicity (Ward, 1964; Khan et al, 1981)(chapter 2.4).

2.2.5 **Class 4**

That there existed a fourth class of antiarrhythmic action was first suggested by Singh and Vaughan Williams (1972(a)) when

studies with the drug verapamil revealed antiarrhythmic effects which could not be attributed to any of the then three classes of action. Since then the calcium channel blocking agents (or calcium antagonists, as they are commonly known) have gained increasing importance not only as antiarrhythmic agents, but also in myocardial ischaemia, systemic and pulmonary hypertension, and hypertrophic cardiomyopathy (Braunwald, 1982).

Since the nodal pathways of the heart are normally depolarized by slow inward currents (chapter 1.1.4), verapamil (the prototype of the class with the greatest antiarrhythmic action) has now became the drug of choice in the management of paroxysmal supraventricular tachycardia (Krikler and Spurrell, 1974; Lazzara and Scherlag, 1980). However calcium antagonists in general have been disappointing in the treatment of ventricular arrhythmia (Lazzara and Scherlag, 1980; Vaughan Williams, 1984). After what has been said about the possible role of slow-response fibres in ischaemic arrhythmias (chapter 1.1.4), this might appear surprising; however, attenuation of ischaemic arrhythmias by calcium antagonists has been described in a number of experimental models (El-Sherif and Lazzara, 1979; Brooks et al, 1980; Curtis et al, 1984) and has been explained on the basis of improved cell-to-cell conduction consequent upon falls in intracellular calcium (Vaughan Williams, 1984).

2.2.6 **Comments on the Vaughan Williams Classification**

As Vaughan Williams himself has written: "the classification is not so much categorization of drugs, in accordance with chemical structures or physical properties, but describes four ways in which abnormal cardiac rhythms can be corrected or prevented" (Vaughan Williams, 1984). It follows therefore that one should not assume that a drug's action is necessarily limited to the class in which it is to be found. Class 1a drugs such as disopyramide and quinidine possess marked anticholinergic effects which are not included in the classification despite profound cardiac effects (Goldstein et al, 1973). The same drugs, by prolonging action potential duration, also possess inherent class 3 activity, a feature also shared by the ß-adrenergic antagonist sotalol (Strauss et al, 1970). It is interesting that, while acute prolongation of action potential duration is not seen with other ß-adrenoceptor antagonists, a similar effect may be observed after long-term ß-blockade in man (Edvardsson and Olsson, 1981), and this has been suggested as the mechanism by which these drugs confer protection from reinfarction and sudden death (Raine and Vaughan Williams, 1976) (chapter 2.4).

So the list could continue with the membrane-stabilizing activity seen in many class 2 drugs (Shanks, 1976) (table 2.3), and the class 4 drug perhexiline (Singh and Nadamanee, 1982). Similarly, electrophysiological studies with amiodarone have revealed features which could include the drug in each of the four antiarrhythmic classes (Bexton and Camm, 1982; Gloor et al, 1983; Mason et al, 1984). More recently, the advent of the specific bradycardic agents

has provided a class of drugs which are thought to act primarily by anion antagonism and thus require separate (class 5) consideration (Millar and Vaughan Williams, 1981).

2.3 Drugs and Sudden Cardiac Death

Although there is a clear relationship between the presence of chronic ventricular ectopic beats and the risk of sudden death, there is little evidence that suppression of ectopy with conventional antiarrhythmic agents has a favourable outcome. Indeed, a number of studies with currently available agents have failed to show any reduction in mortality in controlled trials. May et al (1983) reviewed the results of 14 clinical trials involving 3625 patients with myocardial infarction who were treated with one of four class 1 antiarrhythmic drugs (quinidine, lignocaine, procainamide or disopyramide). Each agent was reported to suppress ventricular arrhythmias during the acute phase of myocardial infarction but none of the trials demonstrated any associated reduction in mortality. Similar results were published by Furberg (1983) in a review of 6 clinical trials with four similar class 1 agents (mexiletine, tocainide, phenytoin, aprindine) and the IMPACT Research Group (1984) which specifically studied the use of a sustained-release preparation of mexiletine. Conversely, however, a number of trials have provided good evidence that the use of ß-adrenoceptor antagonists in the post-infarction period is associated with a reduction in both reinfarction and sudden death

(Multicenter International Study, 1975; Norwegian Multicenter Study Group, 1981; Beta-Blocker Heart Attack Trial Research Group, 1982; Yusuf et al, 1985; The Miami Trial Research Group, 1985; ISIS-1 Collaborative Group, 1986).

The precise mechanism mediating the beneficial effect of ß-adrenoceptor antagonists on sudden cardiac death remains unclear; in particular, there is uncertainty over whether the drugs are acting by a direct antiarrhythmic effect or via a primary anti-ischaemic influence. Although the reduction in mortality cited by the Miami Trial Research Group (1985) was not paralleled by a lower incidence of ventricular fibrillation, other studies have demonstrated significant reductions in ventricular fibrillation with intravenous metoprolol (Ryden et al, 1983) and propanolol (Norris et al, 1984). It is probable that any antifibrillatory/antiarrhythmic effect of the ß-blockers is due to direct antagonism of circulating (and neurogenic) catecholamines, since the drugs have been shown to reverse the lowering of ventricular fibrillation threshold induced by catecholamines in experimental models (Gillis and Clusin, 1984). Furthermore, catecholamine concentrations are known to be abnormally high in the acute infarction period (Richardson, 1963), where they may be inherently arrhythmogenic by nature of the associated increase in myocardial oxygen demand and effects on rate and automaticity. Various metabolic factors have also been held responsible for arrhythmogenesis in the acute infarction period and many of these are consequent upon increased catecholamine activity

(Opie et al, 1979). Hypokalaemia is a particular hazard which may be induced by adrenaline and inhibited by certain ß-adrenoceptor antagonists (Struthers et al, 1981).

Thus the effects of catecholamines and the ß-adrenoceptor antagonists on acute ischaemic arrhythmias must be distinguished from their effects on chronic ventricular arrhythmias, where the drugs are not consistently effective. It follows, therefore, that if the reduction of sudden death afforded by the ß-adrenoceptor antagonists is in fact due to arrhythmia prevention, then it is more likely that the arrhythmias are of a primary, ischaemic nature, rather than those seen with chronic ventricular ectopy.

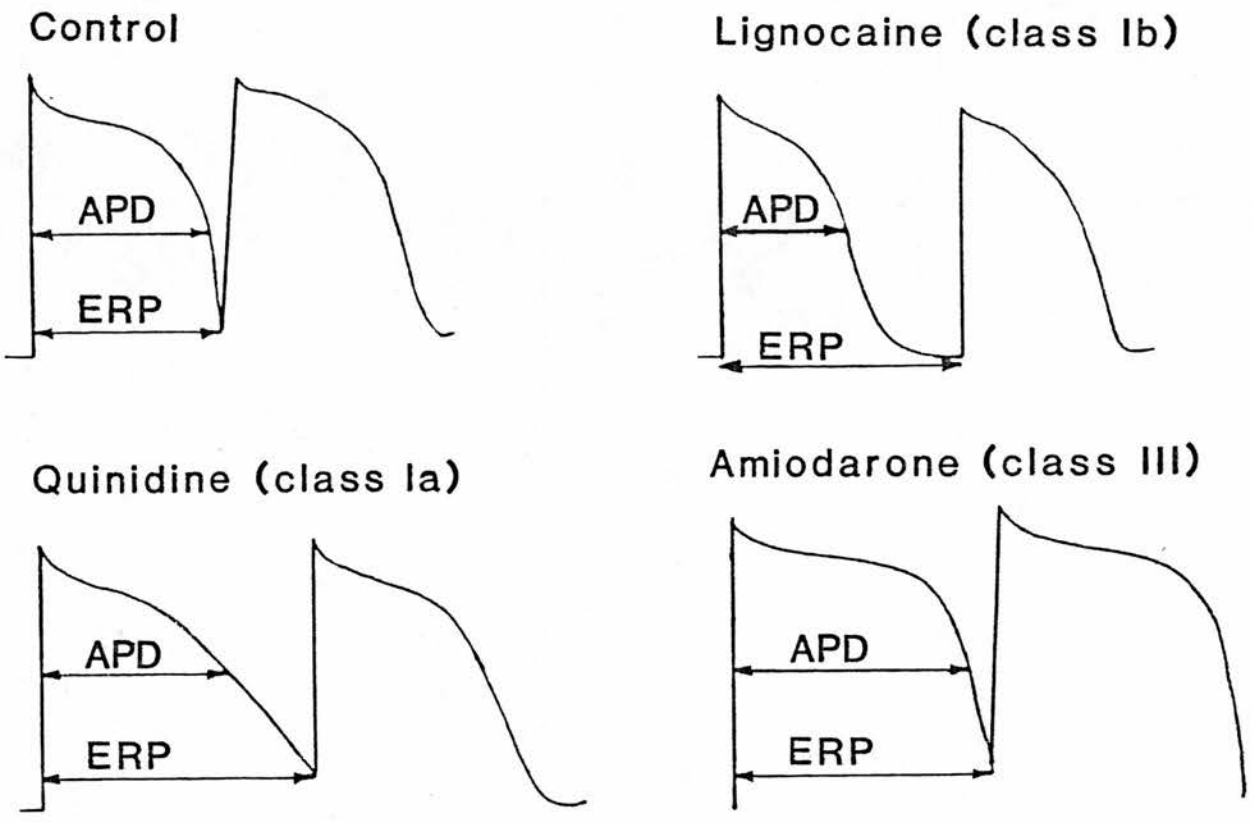


Figure 1.

Changes in the effective refractory period (ERP) relative to the action potential duration (APD) induced by different classes of antiarrhythmic agents.
(Adapted from: Singh and Nadamanee, 1982).

Chapter 3. EXPERIMENTAL MODELS FOR THE EVALUATION OF ANTIARRHYTHMIC DRUGS

So numerous are the experimental models for antiarrhythmic drug assessment that no single classification will adequately cover the subject without considerable overlap. One system, for example, would be to categorize with respect to the initiating arrhythmogenic event, e.g. drug-induced arrhythmias, mechanically or thermally-induced, arrhythmias secondary to CNS stimulation, electrically-induced or those resulting from experimental ischaemia. However, since studies almost exclusively involve mammals (one exception being the occasional use of the toad heart), another classification would be to concentrate on the relative advantages and disadvantages of the respective mammalian species.

The following chapter is intended to give an outline of the differences between the various animal models and the results of studies with drugs from the 4 antiarrhythmic classes. Unfortunately this cannot always be complete as, until fairly recently, few class 3 or 4 agents were known and the degree to which they had been tested in animal models was limited . Nevertheless, an attempt has been made to include this information where possible.

3.1 Non-Canine Models

3.1.1 Isolated Tissues

It has been possible for some time to culture preparations of cardiac cells in such a way as to allow accurate recording of pacemaker activity (Moore et al, 1981). Exposure of such

preparations to arrhythmogenic chemicals such as ouabain or halothane and adrenaline has subsequently allowed evaluation of class 1 and 2 activity respectively (Miletich et al, 1983; Goshima and Wakabayashi, 1983). It could be argued that such a model would obviate the need for animal experimentation, but heart cell cultures are limited in a number of ways: it is believed that any cell line has a tendency to degenerate into a more primitive state when cultured (Miletich et al, 1983). It has furthermore been suggested that cultured heart cells have electrophysiological characteristics more in keeping with pacemaker, as opposed to ventricular, cells (Schanne and Kbaily, 1981). Finally, the study of ischaemic conditions in cell culture preparations has been questioned for its relevance to the clinical situation (Hearse, 1983).

Studies on isolated Purkinje fibres have allowed the accurate determination of electrophysiological parameters such as resting transmembrane potentials, rate of rise of the action potential, maximum rate of depolarization and refractory periods (Weidmann, 1955; Davis et al, 1969; Rosen et al, 1973). Using such preparations, it is possible to study the effects of drugs under physiological conditions, as well as those of altered pH, hypoxia, and changes in ionic concentrations (see chapter 1.1.5). Although in many respects these preparations offer distinct advantages over the cell cultures mentioned earlier, they are subject to similar problems; in particular, it should be remembered that isolated tissues are dependent upon intracellular energy stores, predominantly glycogen.

As these stores are utilized, spontaneous contractions will decline, a phenomenon unrelated to any drug effect.

Use of the 'Langendorff' isolated, buffer-perfused rat heart does not overcome the problem of the slow decline in tissue contractility, but provides a model where it is possible to study the effects of ischaemia on the intact heart (Broadley, 1979; Inque et al, 1984). Using ventricular fibrillation as an endpoint, it has been shown that the addition of class 1 drugs or verapamil to the perfusate offers antiarrhythmic protection (Winslow, 1984); nifedipine, however is less effective and class 2 agents do not appear to be of benefit. Similar studies have also been employed to create conditions of ischaemia by ligating a main coronary artery, and this has proved suitable for a number of antiarrhythmic studies (Lubbe et al, 1978; Inque et al, 1984).

Despite the advantages of the model or apparent efficacy of a drug under test, results of all *in vitro* antiarrhythmic studies will continue to be viewed with caution, since by their very nature they cannot take into account factors such as neurogenic input, humeral influences, biochemical variations, pH fluxes and alterations in arterial gas tensions. In order to gain a better understanding of the true milieu for antiarrhythmic efficacy therefore necessitates the use of intact animals.

3.1.2 Rodents: the Mouse, Rat, and Guinea-Pig

Since rodents are relatively inexpensive, they are particularly

suited to initial screening investigations where serial experiments may be performed with a view to statistical evaluation. Compared with the larger mammals, rodents have comparatively small hearts, and this is the probable explanation for the ability of the animals to defibrillate spontaneously. In the mouse-chloroform model the mechanism of the arrhythmogenic effect of chloroform is not entirely clear, but appears to involve the sensitization of the myocardium to endogenously-released catecholamines (Moore et al, 1981). Although used to assess all classes of antiarrhythmic drugs (Block, 1981), it has been suggested that the mouse-chloroform model is a better indicator of β-adrenoceptor antagonism (Moore et al, 1981).

Infusion of aconitine nitrate into the tail vein of a mouse produces an alternative arrhythmia model which, like the chloroform model, has ventricular fibrillation as the end-point (Dadkar and Bhattacharya, 1974; Nwangwu et al, 1977). The fibrillatory effect of aconitine may be due to its cholinergic actions on the atria, although a direct ventricular action has been suggested (Winslow, 1984). This model appears to be selectively sensitive to drugs with class 1 antiarrhythmic action (Winslow, 1984).

The rat is another frequently used model which has the advantage of providing suitable access for the recording of blood pressure and drawing of venous samples (Kane and Winslow, 1980). Following the studies of Selye et al (1960), the rat rapidly became a standard model for the study of the arrhythmias occurring shortly

after coronary artery ligation where class 1, 2 and 3 agents all appear to have offered antiarrhythmic protection (Kane and Winslow, 1980; Winslow, 1984). More recently a similar model has been described in conscious rats (Curtis et al, 1984) and studies have been extended to include also those arrhythmias which occur upon release of a temporary coronary artery occlusion (reperfusion arrhythmias) (Bergey et al, 1982). There is, however, one fundamental problem when using the rat as an arrhythmia model in that the rat electrocardiogram has no ST segment. Unlike most mammals, the T wave arises immediately following the QRS complex with no isoelectric ST phase (Spear, 1981). Since differences in myocardial transmembrane potentials are likely to be associated with differences in membrane ionic conductances, demonstration of antiarrhythmic efficacy in the rat may not be predictive of a similar action in man. It is known, for example, that doses of digitalis which produce arrhythmias and cardiac disturbances in man have little effect in the rat (Dutta and Marks, 1966), probably as the result of specific differences in sodium/potassium ATPase between the rat and man (Allen and Schwartz, 1969).

Guinea-pigs have most commonly been used as arrhythmia models in the study of arrhythmias produced by toxic doses of cardiac glycosides (Sekiya and Vaughan Williams, 1963; Vaughan Williams and Sekiya, 1963; Hermansen, 1970). In this respect the model is similar to the ouabain-induced arrhythmia in the dog in being most sensitive to the actions of class 1 drugs (see chapter

3.2.1), although the model differs in being sensitive also to the β adrenoceptor antagonists. Like the mouse, the guinea-pig is sensitive to the effects of aconitine; although studied less widely than the former model, results with antiarrhythmic drugs appear similar.

3.1.3 The Rabbit, Cat and Pig

Because of its quiet nature, the rabbit lends itself particularly well to experimentation in the conscious state and for observation by continuous ECG recording. The species has been particularly employed for evaluating effects on nodal conduction, due primarily to the superficial location of both the SA and AV nodes (Wit and Cranefield, 1974). More recently it has been suggested that the rabbit offers a reasonable alternative to rats or dogs for the study of coronary artery occlusion/reperfusion arrhythmias, since it does not share the electrocardiographic anomalies of the rat while at the same time avoiding the increasing cost of canine experimentation (Coker, 1987). Such experiments, however are relatively new and insufficient studies have been carried out to allow a critical appraisal of the effects of the various drug classes.

The cat has been used in the study of arrhythmias due to hypothermia (Rashid and Alps, 1973), glycoside toxicity (Raper and Wale, 1968), central sympathetic stimulation (DiMicco et al, 1977) and the early and late arrhythmias of coronary artery ligation (Ritchie et al, 1979). More recently, a chronic feline myocardial infarction model has also been the subject of investigation

(Myerburg et al, 1977). Of particular interest here is the spontaneous ventricular ectopy which develops one to six months after coronary artery ligation; since this is not a feature of other animal models, it has prompted the suggestion that the chronic feline may approximate more closely to the chronic ectopy seen in man (Myerburg et al, 1977).

A number of investigators have used the pig for electrophysiological, electropharmacological and antiarrhythmic studies (Bergey et al, 1982; Verdouw et al, 1983; Benfey et al, 1984). A unique feature of the porcine model relates to the nature of the coronary circulation; whereas in the dog there exists an extensive collateral circulation, this is much less impressive in the pig, with man occupying a position somewhere in the middle (Bergey et al, 1982; Verdouw et al, 1983). However, there are demonstrated differences between porcine and human Purkinje fibres, and ventricular activation as seen in the porcine electrocardiogram is more rapid (Moore et al, 1981).

3.1.4 **Primates**

As a primate, the monkey would be expected to share similar cardiovascular features with man. Although largely true, there are notable exceptions such as the third main coronary artery in the baboon (Opie et al, 1983). Also the subendocardial nature of experimental infarction in the baboon differs fron the more usual transmural infarction in man (Flameng et al, 1986). The major

disadvantage of using primates, however, relates to expense and the risk of infection, particularly the 'slow' viral encephalopathies which are invariably fatal in man (Opie et al, 1983). Also it should not be forgotten that primates such as the baboon may be particularly vicious and hence not well suited to experimentation in the conscious state.

In summary, therefore, there is no single experimental model which can accurately predict the effectiveness of a potential antiarrhythmic agent in man. Nevertheless, for various practical and financial reasons, it is the dog which has emerged as the most appropriate animal for such studies and will therefore be considered separately. Before leaving this diverse field entirely, however, spare a thought for the unfortunate Japanese quail, which, when fed on a 2% cholesterol diet, developes severe atheroma, myocardial infarction and arrhythmias, and then dies suddenly, and has accordingly been introduced as a further model for sudden cardiac death (Cheung et al, 1983).

3.2 Acute Canine Models

3.2.1 The Ouabain Arrhythmia

Ouabain is one of a number of structurally related, naturally occurring cardiac glycosides which as a group have been shown to decrease resting membrane potentials, decrease the maximal rate of depolarization and overshoot of the action potential, reduce the rate

of repolarization, and enhance phase 4 automaticity (Rosen et al, 1973; Rosen 1985). While it is not clear exactly which of these electrophysiological parameters is responsible for the arrhythmias resulting from toxic doses of ouabain, experimental work has suggested that the arrhythmias result from enhanced automaticity (Vassalle et al, 1963; Rosen et al, 1973) with the pacemaker site located in a left bundle branch or the Purkinje fibre network (Damato et al, 1971). Increasing toxicity appears to be associated with progressive enhancement of such ectopic foci, since at low doses of the drug these changes may only be unmasked when the sinus rate is slowed by vagal stimulation (Vassalle et al, 1963). Again during the early stages of toxicity, the arrhythmias may be dependent upon a fixed relationship with an initiating sinus beat. This has raised the suggestion that the phenomenon is primarily one of triggering (see chapter 1.2.3); however at more advanced stages of ouabain toxicity the ventricular ectopic rhythms lose any such relationship and runs of self-sustaining ventricular tachycardia ensue (Vassalle et al, 1963).

The interaction between ouabain and the autonomic nervous system is a further factor which must be considered in the arrhythmogenic actions of the drug. Like all cardiac glycosides, ouabain has a direct vagal effect (Hoffman and Cranefield, 1960), but studies utilising toxic doses of the drug have indicated an activation of sympathetic ganglia (Konzett and Rothlin, 1952) and stimulation of central sympathetic centres (Standaert et al, 1969).

Since enhancement of phase 4 depolarization is common to both sympathetic stimulation and ouabain toxicity, it was initially suggested that the arrhythmia might be dependent upon catecholamine release (Roberts et al, 1963), but although earlier studies with the β adrenoceptor antagonist pronethalol had demonstrated antiarrhythmic efficacy (Vaughan Williams and Sekiya, 1963), subsequent work by Lucchesi (1965) indicated that this antiarrhythmic effect was not related to pronethalol's β–blocking properties. Pronethalol has significant membrane-stabilizing properties and it is this action which is thought to account for its antiarrhythmic activity in the ouabain model (Somani and Lum, 1965). Indeed, more recent studies with β adrenoceptor antagonists devoid of class 1 activity have shown an exacerbation of the ouabain arrhythmia (Alkondon et al, 1984). While class 1 activity may account for the efficacy of the class 3 agent amiodarone however, it would not, explain the results with the verapamil, a class 4 drug (Caillard and Louis, 1980).

3.2.2 The Halothane-Adrenaline Arrhythmia

It has already been mentioned how myocardial adrenergic stimulation results in a number of potentially arrhythmogenic electrophysiological changes, largely mediated by β receptors (see chapter 1.1.5). Whilst this is rarely of clinical importance in otherwise healthy individuals, it was recognised many years ago that these arrhythmogenic properties could be greatly enhanced by

prior exposure to general anaesthetic agents, often with catastrophic consequences (Meek, 1941). It was this observation which led to the experimental models where arrhythmias could be produced by administering intravenous adrenaline to dogs respired with cyclopropane (Nickerson and Nomaguchi, 1949), chloroform (Vick, 1966) and more recently halothane (Price and Ohnishi, 1980).

Although it is believed that the arrhythmogenic effects of adrenaline reflect its actions in enhancing phase 4 automaticity in the conducting network (Hoffman and Singer, 1967), it is less clear why potentiation should be apparent with anaesthetic agents: while some such agents (eg. diethyl ether) are capable of stimulating sympathetic outflow, others (including halothane), result in lower levels of circulating catecholamines (Roizen et al, 1974). However, all general anaesthetics produce direct myocardial depression by an inherently arrhythmogenic mechanism thought to involve a reduction in the amount of free calcium available for the contractile elements (Price and Ohnishi, 1980).

The haemodynamic conditions pertaining at the time of arrhythmogenesis have also been the subject of considerable debate: it has been suggested that a pressor response to adrenaline is a prerequisite before the emergence of the arrhythmia and may even dictate the severity of the arrhythmia (Dresel and Sutter, 1961). Heart rate may be similarly important: a number of early experiments utilizing both vagal stimulation (Dresel and Sutter, 1961; Vick, 1966) and atrial pacing techniques (Dresel et al, 1960)

demonstrated that a positive chronotropic response was necessary before the emergence of the arrhythmia, but more recently it has been shown that the arrhythmia may not be dependent so much upon a tachycardia in response to adrenaline as upon an initial bradycardia induced by halothane (Hashimoto and Hashimoto, 1972). Halothane is known to have a negative chronotropic effect on the sinus node which is resistant to atropine (Reynolds et al, 1970). Thus it appears that the mechanism of sensitization by halothane to produce multifocal ventricular ectopics and tachycardia may relate to a slowing of sinus rate by halothane in conjunction with a pressor response and tachycardia from the adrenaline. The ventricular fibrillation occasionally seen is thought to be independent of heart rate and may relate primarily to the depressant action of halothane on the myocardium (Price and Ohnishi, 1980).

Not surprisingly, the halothane-adrenaline model has been used extensively as an index of the β adrenoceptor blocking properties of antiarrhythmic agents. However, in a recent study investigating the effects of prazosin and metoprolol on this arrhythmia, it was discovered that β_1 blockade from metoprolol was significantly less effective than α_1 blockade afforded by prazosin (Maze and Smith, 1983); nor was the effect of prazosin related solely to haemodynamic consequences as sodium nitroprusside was found to be ineffective in abolishing the same arrhythmia despite producing a similar fall in blood pressure and attenuating the pressor response to adrenaline. Calcium channel blockers have also been found

effective in this model, although variable reports have resulted from the testing of class 1 agents (Shibuya et al, 1963).

3.2.3 The Early and Late Arrhythmias of Coronary Artery Ligation

The first example of experimental coronary artery ligation may date as far back as 1698 when Chirac was reported to have tied the coronary artery of a dog (Porter, 1894). The animal, however, died shortly afterwards and since the physicians of the day had little appreciation of the importance of the coronary vasculature, interest remained quiescent until Erichsen (1842) reported the effects of coronary artery ligation on heart rate and rhythm. This was followed by a number of similar experiments in various animal species, but it was not until 1918 that the results of such manoevres could be corroborated with simultaneous electrocardiographic recording (Smith, 1918). These and subsequent investigations have shown that acute occlusion of a major coronary artery is associated with a high incidence of severe ventricular arrhythmic activity and death within the first few minutes of occlusion (Dreifus et al, 1981). More recently, 2 distinct periods of maximal activity have been identified (Kaplinsky et al, 1979): immediate ventricular arrhythmias (IVA, or 1a) occur from 2-10 min after coronary occlusion and are thought to relate to reentrant mechanisms in the subendocardial region of the ischaemic zone (Kaplinsky et al, 1979). Delayed ventricular arrhythmias (DVA, or 1b)

occur from 12-30 min after ligation; while they, too, are probably dependent on reentry , experimental evidence has suggested that the mechanisms may not parallel those responsible for the immediate arrhythmias (Menkin et al, 1979; Russell et al, 1984).

Following the discovery that coronary artery ligation in conscious dogs was associated with a mortality of up to 3 times that seen in anaesthetised animals (Manning et al, 1939), McEachern and coworkers (1940) demonstrated that prior thoracic sympathectomy could reduce mortality in conscious dogs from 75% to 10%. Furthermore, it has been shown that similar reductions in mortality (unrelated to the area of infarction) can be observed after cardiac denervation in anaesthetised dogs if sufficient time is allowed for myocardial catecholamine depletion, emphasizing the role of both endogenous and neurogenic catecholamines in the genesis of the acute arhythmias (Ebert et al, 1970). Alpha adrenoceptor antagonists have proved successful in attenuating these arrhythmias in a number of models (Sheridan et al, 1980; Penny and Sheridan, 1982; Benfey et al, 1984) and similar results (although less dramatic) have been obtained with at least one of the β adrenoceptor antagonists (Gamble and Cohn, 1972). Using various manipulations of the basic acute model, various investigators have reported benefit from the use of procainamide and quinidine (Stephenson et al, 1960), bretylium (Kniffen et al, 1975), aprindine (Verdouw et al, 1977), lignocaine (Gamble and Cohn, 1972) and verapamil (Fondacaro et al, 1978). However, the relative advantages

of each of these drugs is fairly small and none appears optimally protective, possibly due to the variable incidence of ventricular fibrillation within any group under study (Dreifus et al, 1981).

Since the major determinant of infarct size is the period of time during which tissue is rendered anoxic, reperfusion of the area of myocardium at risk has for some time been a primary goal of many cardiologists. It is interesting, therefore that while (from an arrhythmia point of view) this is a fairly safe procedure clinically (Witkowski and Corr, 1984), in experimental models reperfusion after a variable period of coronary artery occlusion is associated with serious ventricular arrhythmias (Battle et al, 1974) and is now included as one of the most malignant arrhythmia models (Dreifus et al, 1981). Although evidence suggests that reperfusion arrhythmias share the same reentrant basis as the arrhythmias of acute ischaemia (Scherlag et al, 1970), it has been postulated that the site of origin of the arrhythmias may not be the same in both models (Agarwal et al, 1984). In general, drugs with α adrenergic blocking properties or class 1 activity appear to be most useful in controlling the arrhythmias associated with reperfusion; class 4 drugs have also been favourably reported but β adrenergic blockade does not appear to be protective (Sheridan et al, 1980; Brooks et al, 1980; Dreifus et al, 1981; Thandroyen et al, 1983; Winslow, 1984).

In 1950, Harris noted "previous experimental studies on ventricular ectopic activity following coronary occlusion ... have

been confined to observations made within a brief acute period after occlusion ... The scarcity of experimental studies upon the delayed development of ventricular arrhythmias ... is attributable in part to the high rate of early mortality via ventricular fibrillation which results from abrupt occlusion ... of the left coronary artery." The subsequent description of Harris's two-stage ligation allowed the identification of a 'late' ventricular arrhythmia which has been regarded for many years as one of the most valuable experimental arrhythmia models.

The technique originally described involved the application of a critical stenosis, or partial occlusion, of the left anterior descending coronary artery, followed 30 minutes later by complete occlusion. Any acute arrhythmic activity was noted to cease totally within 4 hours, but around 8 hours a second arrhythmic phase began which peaked at 24-48 hours and subsided completely by the fourth day (Harris, 1950). The arrhythmia itself is characterized by a rapid multifocal ventricular tachycardia interspersed with occasional sinus beats; it is of note that ventricular fibrillation is not a feature of this particular model.

The origin of the '24 hour' arrhythmia is thought to arise from enhanced automaticity in surviving subendocardial fibres (Friedman et al, 1973; Scherlag et al, 1974; Horowitz et al, 1976). Evidence for the automatic nature of the arrhythmia has been demonstrated by overdrive pacing techniques (Scherlag et al, 1974; Horowitz et al, 1976) and it is interesting to speculate on the observation that the

arrhythmia may be entirely absent in dogs which are particularly ill postoperatively (Harris, 1950); presumably this relates to a form of overdrive suppression by a rapid sinus node discharge rate.

It has been suggested that drugs from each of the antiarrhythmic classes are effective to a greater or lesser degree in abolishing the arrhythmia occurring 24 hours after coronary ligation in dogs (Caillard and Louis, 1980; Dreifus et al, 1981; Winslow, 1984). Although some have postulated that the arrhythmia may be subject to autonomic control (Martins, 1985), studies with α agonists and antagonists have shown little effect (Constantin and Martins, 1987) and results with β adrenoceptor blockade remain equivocal; moreover, as with the ouabain-induced arrhythmia, reported benefit from the use of β adrenoceptor antagonists may relate primarily to class 1 activity (Allen and Shanks, 1974; Hashimoto et al, 1982).

It has been suggested that this particular arrhythmia model may resemble the sequence of events seen after acute myocardial infarction in man (Davis et al, 1982). Survival of subendocardial Purkinje fibres after infarction has been demonstrated in man (Fenoglio et al, 1976) but although this may be a site of arrhythmia genesis, the essentially benign nature of the experimental model distinguishes it from the clinical situation.

Finally a number of alternative methods have been described for the production of experimental myocardial ischaemia and/or

infarction. These include the use of ameroid constrictors upon, or balloon occluders within, the coronary vasculature (Coltart et al, 1974; Rosenfeld et al, 1978) and the radiologically-guided introduction of an occluding bead or beads into the appropriate coronary vessel (Wilkerson and Downey, 1978; Karagueuzian et al, 1982). While some of these methods may allow the study of acute myocardial ischaemia in the conscious animal, there does not appear to be any advantage in studies of the '24 hour' arrhythmia. Indeed, without the facility of direct inspection of the coronary vasculature, it would be difficult to assess the degree of collateral circulation in deciding a point of occlusion.

3.2.4 The Adrenaline-Induced Arrhythmia, 3-5 days after Coronary Artery Ligation

While there may be controversy over the role of sympathetic factors in the arrhythmia occurring 24 hours after coronary ligation, it has been demonstrated that the infarcted myocardium is more sensitive to the effects of exogenously administered adrenaline for several days after ligation. Thus adrenaline, in doses which cause minimal effects on rhythm in normal dogs, is capable of producing a prolonged ventricular tachycardia in post-infarcted animals (Maling and Moran, 1959). More recently, studies with isolated Purkinje fibres 24 hours after infarction have confirmed this enhanced sensitivity to the arrhythmogenic effects of adrenaline, particularly in fibres from the infarcted areas (Cameron et al, 1982).

In their original study, Maling and Moran (1959) concluded that the factors necessary for the production of the arrhythmia included direct stimulation of the myocardium and an associated slowing of sinus rate. This explains the arrhythmogenic actions of agents such as adrenaline and noradrenaline while isoprenaline, despite its stimulant properties, increases the sinus rate both directly and secondary to the fall in blood pressure, and is ineffective in establishing the arrhythmia. In many respects, therefore, the model has similar characteristics to the halothane-adrenaline model, although it may be argued that the latter is not as relevant to the clinical situation as the conscious, post-infarcted animal with a high circulating catecholamine load. A summary of the factors thought to be necessary for arrhythmia genesis in the respective adrenaline-induced arrhythmia models is given in figure 3.1.

Although not studied as widely as the halothane-adrenaline model (there are no records of experiments investigating class 3 or 4 drugs), the adrenaline-induced arrhythmia after coronary artery ligation appears to share the same features when used as a test of antiarrhythmic activity. Thus the greatest efficacy has been demonstrated with the β adrenoceptor antagonists, while class 1 agents in general appear ineffective (Allen et al, 1974).

3.3 Chronic Canine Models

3.3.1 The History of Electrophysiological Studies

The first recorded experiment which investigated the effects of electricity on the heart appears to have been in the 1700's when Abilgard shocked a chicken lifeless using current stored in a Leyden jar (Abilgard, 1775). In 1889 two Swiss physiologists, Prevost and Battelli, demonstrated that electric currents produced ventricular fibrillation when applied directly to the heart (Prevost and Battelli, 1889). It is of note that the reciprocal finding of resuscitation by similar electric shocks was added only as a footnote. Such studies laid the foundation for what remains to this day a valuable electrophysiological investigation, ie. the ventricular fibrillation threshold (VFT).

The VFT requires the application of electric current (either as a single, critically timed pulse or as a train of pulses) to the ventricular myocardium during the vulnerable period of recovery from a previous depolarization (Moore and Spear, 1975). The fibrillation threshold value (the minimum intensity of current required to produce ventricular fibrillation) is considered to be an index of ventricular vulnerability to fibrillation, and the ability of test agents to elevate this threshold is generally accepted as an indication of potential antiarrhythmic activity (Lynch and Lucchesi, 1987). It is well established that the VFT is lowered during acute myocardial ischaemia and infarction, reflecting the greater propensity of the ischaemically injured heart to fibrillate. In a recent study with chronic post-infarction dogs, a reduction in VFT was found in those animals with inducible sustained ventricular

tachycardia (Gang et al, 1982), suggesting a link between traditional measures of ventricular vulnerability to fibrillation and the inducibility of ventricular arrhythmias by programmed electrical stimulation.

3.3.2 Programmed Electrical Stimulation in Chronic Canine Models of Myocardial Infarction

Until fairly recently, it was believed that since animals did not exhibit spontaneous ventricular ectopy much later than 3-4 days after experimental coronary artery occlusion (an exception being the cat-see chapter 3.1.3), a suitable chronic model of myocardial infarction did not exist. However, in a series of studies in dogs, El-Sherif and co-workers were able to demonstrate delayed, slowly-conducting electrical activity within areas of damaged myocardium, 3 to 5 days after experimental coronary artery occlusion (El-Sherif et al, 1977 (a),(b)). When such activity became continuous, as occurred during rapid pacing or with the induction of appropriately timed premature beats, runs of ventricular tachycardia ensued, prompting the authors to suggest a reentrant basis. It is interesting that similar observations had previously been made in acute canine myocadial infarction models (Waldo and Kaiser, 1973; Boineau and Cox, 1973) and were subsequently to be confirmed clinically (Josephson et al, 1978 (a),(b)).

At around the same time as these observations were made, a number of independent investigators were able to demonstrate

induction of ventricular tachyarrhythmias in post-infarcted dogs when challenged with programmed electrical stimulation (Glassman et al, 1978; Karagueuzian et al, 1979; Michelson et al, 1980(a); Garan et al, 1980; Gibson and Lucchesi, 1980; Patterson et al, 1980). These experiments were all based on the finding that in the post-infarcted canine heart, electrical stimuli subthreshold for ventricular fibrillation could more easily induce ventricular tachyarrhythmias when delivered as two or more closely coupled stimuli (Thompson and Lown, 1972). Furthermore, more recent studies have shown that the addition of a second and third extrastimuli will also result in a progressive shortening of refractoriness, permitting the propagation of ectopic beats much earlier during the cardiac cycle than can be achieved with one extrastimulus alone (Moore et al, 1986).

Following the earlier finding that release of a coronary artery occlusion after a period of 40 minutes to 3 hours was capable of salvaging 'islands' of surviving myocardium (Maroko et al, 1972; Ginks et al, 1972; Reimer et al, 1977), a number of investigators now routinely include reperfusion in their surgical protocols. This is based on the proposal that these areas of surviving myocardium offer a better substrate for the genesis of reentrant pathways, although with the exception of one trial (Karagueuzian et al, 1979) this does not appear to have been studied under controlled conditions.

To date, numerous antiarrhythnic agents representing the

various classes of antiarrhythmic drug action have been evaluated in chronic coronary artery occluded or occluded/reperfused preparations, either in the conscious or anaesthetised state. The efficacy of these agents in suppressing the arrhythmias induced by programmed electrical stimulation is summarized in tables 3.1 and 3.2. In general, class 1 agents have been only marginally effective in suppressing these arrhythmias; in fact, several studies have shown significant proarrhythmic effects (Patterson et al, 1980; Patterson et al, 1982 (a); Zimmerman et al, 1985; DiCarlo et al, 1985) (also see chapters 3.3.4 and 11.3). The effects of beta-adrenergic antagonists have varied widely with the agent under test: results with metoprolol and nadolol have been disappointing, while propanolol, timolol and sotalol have each demonstrated significant antiarrhythmic activity (see table 3.1). Variable results, too, have been reported with the class 3 agents, although this may in part be due to the lack of a 'pure' class 3 drug, devoid of ancillary electrophysiological effects (Lynch and Lucchesi, 1987). Results with class 4 drugs tend to suggest that the calcium antagonists are ineffective in these models (see table 3.2).

Programmed electrical stimulation in man was originally introduced as a means of studying the electrophysiological events associated with ventricular tachycardia (Wellens et al, 1972,1974). Subsequently it has been employed as a means of directing drug therapy in the management of patients with recurrent ventricular arrhythmias (Bigger et al, 1982; Podrid et al, 1983; Echt and Mason,

Drug		Preparation	Effects on Induced VT
Lignocaine	(i)	C/R	0/8 suppressed:VT rate slowed
	(ii)	(A+C)/R	VT facilitated
Procainamide	(i)	A/R	5/9 suppressed: VT rate slowed
	(ii)	C/R	0/5 suppressed: VT rate slowed
Phenytoin		A/O	2/10 suppressed: VT rate slowed
Quinidine		C/R	4/8 suppressed: VT rate slowed
Encainide		C/R	0/5 suppressed
Flecainide	(i)	A/R	1/9 suppressed: Proarrhythmic effects
	(ii)	A/R	0/7 suppressed: VT facilitated
Propanolol	(i)	C/R	5/5 suppressed
	(ii)	A/R	9/15 suppressed
Timolol		A/R	13/22 suppressed
Metoprolol		C/R	1/14 suppressed: VT rate unchanged
Sotalol	(dl)	C/R	5/9 suppressed: VT rate slowed
	(l)	C/R	10/14 suppressed: VT rate slowed
	(d)	C/R	13/22 suppressed: VT rate slowed
Nadolol		C/R	0/7 suppressed: VT rate unchanged

Table 3.1

Programmed Electrical Stimulation in Chronic Canine Models of Myocardial Infarction:
1. Effects of Class 1 and 2 Drugs.

(Key: A, anaesthetised: C, conscious: VT, ventricular tachycardia
O, occlusion: R, occlusion/reperfusion.)
(Adapted from: Lynch and Lucchesi, 1987)

Drug		Preparation	Effects on Induced VT
Bretylium		C/R	7/12 suppressed (delayed): Proarrhythmic effects noted
Bethanidine		C/R	2/13 suppressed: Proarrhythmic effects
Meobentine	(i)	A/R	6/6 suppressed
	(ii)	C/R	0/9 suppressed
Verapamil	(i)	A/O	0/5 suppressed: VT rate unchanged
	(ii)	C/R	0/6 suppressed: VT rate unchanged
Diltiazem		C/R	0/7 suppressed: Proarrhythmic effects
Bepridil		(A+C)/R	4/12 (low dose): 9/19 (high dose)

Table 3.2

Programmed Electrical Stimulation in Chronic Canine Models of Myocardial Infarction: 2. Effects of Class 3 and 4 Drugs.

(Key: A, anaesthetised: C conscious: VT, ventricular tachycardia
O, occlusion: R, occlusion/reperfusion.)
(Adapted from: Lynch and Lucchesi, 1987)

1984; Kim et al, 1986).

Numerous studies have utilised various programming techniques in an effort to predict those at risk of sudden death after myocardial infarction. While some have found programmed stimulation of valuable prognostic significance (Horowitz et al, 1978; Swerdlow et al, 1983; Stevenson et al, 1985; Waspe et al, 1985), others have questioned its applicability (Marchlinski et al,

1983). Furthermore, it remains unclear if inducibility of arrhythmias by programmed stimulation might not be an independent risk factor (Friedman and Yusuf, 1986). Roy and coworkers (1985(a)) studied the use of programmed stimulation as a guide for pharmacological therapy in survivors of cardiac arrest (a group at high risk of recurrent sudden death). Results indicated a 12% recurrence rate (9-20 month follow-up) compared with 48% in patients treated empirically. However, in the same studies up to 40% of patients failed to respond to stimulation; recurrence of sudden death in these non-responders was as high as 32% (Roy et al, 1985 (b)). Thus in a significant proportion of survivors of cardiac arrest, programmed stimulation alone will be ineffective in identifying risk and guiding therapy.

3.3.3 The Conscious Canine Model of Sudden Death

In 1982, Lucchesi and co-workers described a chronic canine model of myocardial infarction which reliably responded to an additional ischaemic insult at a distant site with the development of ventricular fibrillation (Patterson et al, 1982 (b)). The procedure involved the insertion of a silver wire electrode into the lumen of the left circumflex artery at the same time as the left anterior descending artery was subjected to a 2-hour occlusion followed by reperfusion. After electrophysiological testing and programmed stimulation on the third or fourth postoperative day an anodal current of 150mA is applied to the wire. This results in a sequence

of events, incluing arterial intimal damage, platelet aggregation, oscillations of blood flow, electrocardiographic evidence of ischaemia and, ultimately, the development of ventricular fibrillation in over 90% of cases (Wilber et al, 1985). It is notable that similar ischaemic insults in dogs without antecedent infarctions, while in many cases resulting in posterolateral infarctions, do not generally result in ventricular fibrillation (Patterson et al, 1982 (b)). This suggests that lethal arhythmias are more likely to arise in peri-infarcted tissues, electrically deranged by the infarction process and rendered more sensitive to the effects of ischaemia, a point made previously in a similar chronic canine model (Kabell et al, 1984).

3.3.4 Proarrhythmic Effects of Antiarrhythmic Drugs

Despite the demonstrated efficacy of many antiarrhythmic drugs in suppressing ventricular ectopy, we have already seen that this may not be paralleled by a reduction in the incidence of sudden death. Indeed, a number have studies have shown that between 30 and 60 per cent of patients resuscitated from cardiac arrest have been taking antiarrhythmic drugs at the time of arrest (Liberthson et al, 1974; Moss et al, 1977; Ruskin et al, 1983). The ability of antiarrhythmic drugs to exacerbate or induce arrhythmias has been considered since it was first shown that syncopal attacks during quinidine treatment might be related to paroxysms of ventricular flutter or fibrillation (Selzer and Wray, 1964). However, direct

evidence has been difficult to obtain because of the inherent unpredictability of many arrhythmias.

In a retrospective analysis of antiarrhythmic drug action, Velebit et al (1982) assessed 24-hour Holter monitoring and maximal exercise stress testing in 155 consecutive patients referred because of ventricular arrhythmias: in a total of 722 drug tests, a worsening of arrhythmia (defined as the occurence of a four-fold increase in the frequency of ventricular premature complexes, a ten-fold increase in repetitive forms, or the first emergence of sustained ventricular tachycardia coincident with the time course of action of the drug under study) was observed in 80, or 11.1%. Exacerbation was apparent with each of the 9 drugs studied (quinidine, procainamide, disopyramide, propanolol, metoprolol, aprindine, mexiletine, tocainide and pindolol) and it is significant that blood concentrations of the drugs were consistently within the therapeutic range.

It has already been mentioned (chapter 1.2.3) how the genesis of a reentrant circuit is dependent upon a critical balance of conduction and refractoriness. Thus it may be seen how a drug with a preferential effect on one of these parameters (eg. conductivity) might suppress arrhythmias in one situation (by extinguishing slowly-moving wavefronts in a reentrant pathway) while exacerbating arrhythmias in another (by sufficiently slowing the wavefront of depolarization to allow recovery within a reentry pathway). In the case of the local anaesthetic agents, it has been

shown that the effects of lignocaine on ischaemic myocardial fibres may be different from its effects on normal areas (Allen et al, 1978), and this may predispose to local block of impulse propagation and fractionation of the wavefront, leading to the creation of multiple reentrant circuits (Hoffman and Dangman, 1986).

The same arguments used above with respect to conduction may also be employed when considering refractoriness: thus while prolongation of the refractory period may abort reentrant circuits by rendering refractory those tissues which constitute the abnormal pathway, the same electrophysiological effects may allow the propagation of the wavefront by an alternative, more slowly-recovering area of myocardium. In particular, this may explain the possible proarrhythmic effects of the class 1a and class 3 drugs. Special mention should also be made of the proarrhythmic effects of the class 1c agents flecainide and encainide (Winkle et al, 1981; Olsson and Edvarsson, 1981). These drugs have shown inordinate propensity to convert ventricular ectopy to sustained arrhythmias and even ventricular fibrillation. Furthermore, in many cases the resulting arrhythmias may be remarkably resistant to defibrillation (Winkle et al, 1981; Duff et al, 1982).

Another potentially proarrhythmic electrophysiological drug action is prolongation of the QT interval, which has been shown to lead to ventricular tachycardia and torsade de points (Khan et al, 1981).This is particularly likely to occur with inhomogeneous prolongation of the action potential duration, such as occurs with

the class 1a drugs, and is compounded in conditions in which drug delivery and uptake varies from region to region as the result of disease processes (Goldstein et al, 1984).

Other possible arrhythmogenic effects of drugs may not necessarily be related to electrophysiological properties. Anticholinergic effects of drugs such as quinidine and disopyramide are known to favour ventricular electrical instability in the presence of ischaemia (Goldstein et al, 1973), and reflex sympathetic stimulation might be expected of any drug with negative inotropic activity. Negative inotropes themselves may be inherently proarrhythmic by exacerbating cardiac failure and such deleterious haemodynamic effects may also prejudice recovery from ventricular fibrillation (Euler et al, 1986).

Many of the early proarrhythmic studies used either Holter monitoring or exercise stress-testing with an increase in the number of ventricular ectopic beats as an end-point. However, recent evidence suggests that in some cases invasive electrophysiological testing may unmask latent proarrhythmic effects which are not apparent with simple electrocardiographic monitoring (DiBianco et al, 1982). In one study (Duff et al, 1982) chronic ventricular ectopy (as assessed by ambulatory monitoring) was actually improved by the same drug (encainide) which demonstrated proarrhythmic effects with programmed electrical stimulation. Furthermore, with electro- physiological testing, a defined inducible arrthythmia serves as the basis for comparing all subsequent results, drug

exposure is brief, blood levels are defined throughout and adverse effects can be treated appropriately and promptly (Goldstein et al, 1984).

A summary of some of the larger clinical studies which have used programmed electrical stimulation as an investigational tool for evaluating proarrhythmic responses to antiarrhythmic drugs is given in table 3.3. It can be seen that most of these studies have involved the class 1a and 1c drugs, although results are available for class 1b (Poser et al, 1985), class 2 (Poser et al, 1985), class 3 (Rae et al, 1985) and class 4 (Torres et al, 1985).

In a thorough study of 181 patients referred because of a variety of ventricular arrhythmias, Torres et al (1985) discontinued all drugs and found that ventricular tachycardias (VT) could be induced by programmed stimulation in all patients. After subsequent drug dosing, a proarrhythmic response was defined as:

(a) spontaneous development of VT coincident with the administration of the drug.

(b) a reduction in the number of stimuli needed to induce VT.

(c) development of sustained VT where only non-sustained VT had previously been induced.

(d) VT requiring cardioversion which previously had terminated spontaneously or with burst pacing.

(e) Induction of VT after drug dosing when previously no VT had been induced.

Reference	Drug	Arrhythmia History	Criteria for Proarrhythmic Response	Positive Results
Duff et al (1982)	E	History of NS-VT	Enhanced ease of induction	4/6
DiBianco et al (1982)	E	Frequent VPC's	Induction of VT	3/20
Rinkenberger et al (1982)	D,A,E,Q	History of NS-VT	Conversion to S-VT	11/83
Ruskin et al (1983)	Q,D	Survivors of VF	Induction of VT	4/6
Poser et al (1985)	Various	History of NS-VT	Conversion to S-VT Fewer stimuli for NS-VT	3/216† (trials)
Rae et al (1985)	Various	Inducible VT	Conversion S-VT to VF or NS-VT to S-VT	25/384 (trials)
Torres et al (1985)	Various	Inducible VT	Various (see text)	43/181
Buxton & Josephson (1986)	Various	Inducible NS-VT	Conversion to S-VT	6/36
Au et al (1987)	Q,D,P	Frequent VPC's	Induction of VT	6/24

†Drug testing with multiple agents caused aggravation of arrhythmia in 19/63 patients.

Table 3.3

Results of previous proarrhythmic studies with programmed electrical stimulation in patients.

(Key: E, encainide: D, disopyramide: A, amiodarone: Q, quinidine: P, procainamide. VF, ventricular fibrillation: S-VT, sustained ventricular tachycardia. NS-VT, non-sustained ventricular tachycardia: VPC, ventricular premature beat.)

It is notable that the authors conclude "there appears to be no way of predicting which patients will have an arrhythmogenic response to a given drug". In particular, for the group as a whole, there were no significant changes in the PR interval, QRS duration, QT_c or refractoriness. Also, despite a range of proarrhythmic effects from 28% for ethmozine to 8% for procainamide and bepridil, further analysis showed no statistical difference between the drugs tested.

In a study which confirmed a proarrhythmic response in 6 of 24 patients tested, Au et al (1987) found that these patients did not differ significantly from the other 18 except with respect to refractory periods which tended to be shorter, and the incidence of digoxin usage which was significantly higher in the proarrhythmic group. The latter finding may be of particular interest since digitalis- related compounds had been implicated as having proarrhythmic potential in earlier studies, both clinical (Moss et al, 1981; Bigger et al, 1981) and experimental (Lynch et al, 1986).

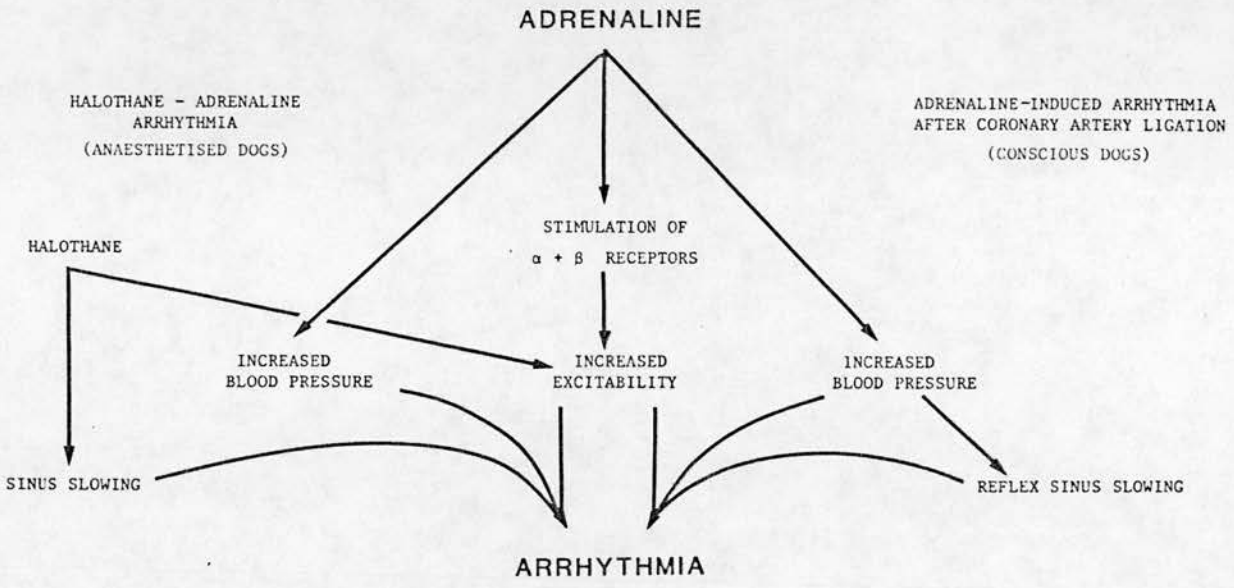


Figure 1.

Possible explanation for the arrhythmogenic actions of adrenaline in anaesthetised dogs respired with halothane and in conscious dogs following experimental coronary artery occlusion (CAL). In the former, sinus slowing is produced by a direct effect of halothane on the sinus node; in the conscious dog following CAL, sinus slowing is secondary to the pressor effect of adrenaline and is mediated by intact autonomic reflexes.

Chapter 4. METHODS

All experimental animal work described in the following chapters was carried out in accordance with, and under the jurisdiction of, the appropriate Government legislation. During his tenure of a DHSS (NI) Research Fellowship, the author was in receipt of animal license types A, B, C, E and EE.

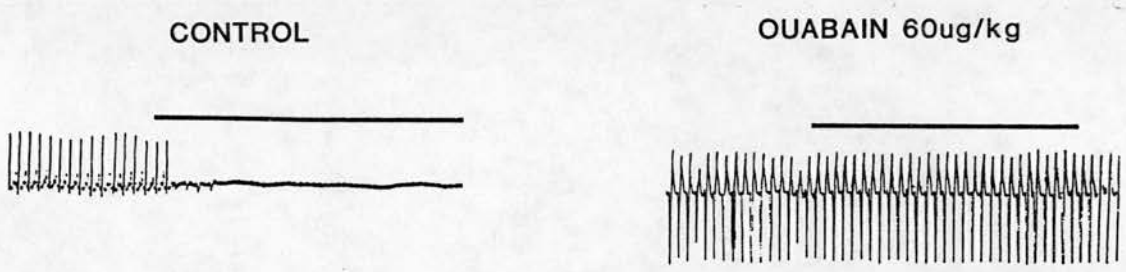
Without exception, all experiments involved the use of healthy adult greyhounds (both sexes) cared for at the Queen's University Medical Research Unit, New Forge Lane, Belfast.

4.1 Acute Models

Ouabain-induced arrhythmias

Observation were made in adult greyhounds of both sexes. The dogs were anaesthetized by the intravenous administration of sodium pentobarbitone (30 mg/kg), intubated and ventilated with room air at a rate of 18/min and tidal volume of 13 ml/kg/min (Palmer Ideal Pump). Lead II of the electrocardiogram (ECG) and arterial pressure (recorded by means of a cannula in the femoral artery) were simultaneously displayed on an oscilloscope screen and recorded on a Lectromed LX 216 2-channel recorder. An additional cannula in the femoral vein served for the administration of drugs. The right vagus nerve was exposed in the neck and divided between ligatures. A bipolar electrode was applied to the distal end of the nerve, which was stimulated for periods of 10 sec with shocks of 1 msec duration at a frequency of 25 Hz and a voltage sufficient to cause maximal slowing of the heart rate without loss of sinus dominance (0.2-3.5 V). Ventricular tachycardia was produced by the intravenous administration of ouabain 40 µg/kg, followed 15 min later by 20 µg/kg and then 10ug/kg every 15 min until a ventricular arrhythmia was produced. Stimulation of the vagus nerve with the previously established voltage was repeated to confirm the ventricular origin of the arrhythmia (figure, over). With the arrhythmia established for 10

min, increasing intravenous doses of drug(s) were administered until either the return of sinus rhythm or the death of the dog. An example of the ouabain-induced arrhythmia is given in figure 4.1.



——— corresponds to stimulation of the right vagus nerve.

Halothane-adrenaline arrhythmias

Adult greyhounds were anaesthetized by the intravenous administration of pentobarbitone (30 mg/kg). Ventilation, measurement of blood pressure and electrocardiographic recording was similar to the methods employed for the ouabain-induced arrhythmia. Following preparation, halothane was introduced at a concentration of 1% in room air. After 15 min, increasing doses of adrenaline were administered from 0.4 µg/kg at 0.4 µg/kg increments; these were continued at 10 min intervals until the production of ventricular tachycardia or multifocal ventricular ectopic beats .(figure 4.2). If ventricular fibrillation occurred,

halothane administration was discontinued and an external DC counter shock was applied (50-100 Joules: Pantridge Defibrillator type 280) (figure 4.2). Halothane was then reintroduced and the protocol continued with a lower dose of adrenaline after a 15 min period. After determination of a test dose of adrenaline which could produce a similar, severe arrhythmia on 2 successive occasions, increasing doses of drug(s) were administered at 10 min intervals. 5 min after each dose of the drug(s) under study, the dog was rechallenged with the test dose of adrenaline. The experiment continued until the test dose of adrenaline failed to produce any ectopic response.

Arrhythmias following acute coronary ischaemia

Adult greyhounds were anaesthetized by the intravenous administration of sodium methohexitone (10 mg/kg), intubated and respired with a 1.5% concentration of halothane in room air. Lead II of the electrocardiogram and blood pressure (recorded by means of a cannula in the femoral artery) were monitored continuously on a Lectromed LX 216 2 channel recorder. Following thoracotomy, the fourth or fifth left rib was dissected free and removed and the heart exposed. The anterior descending branch of the left coronary artery was then dissected free and a ligature passed loosely behind the artery. After a 5 min control period the dog was randomly allocated to receive intravenous placebo or drug(s). Five min later a critical

stenosis of the artery was produced by tying the ligature tightly around the artery and a 21-gauge needle, which was then withdrawn. Following ligation, the total number of ventricular ectopic beats were counted at 5 min intervals over a 30 min period. An example of these arrhythmias is given in figure 4.3.

Arrhythmias 24 hours after acute coronary artery ligation

Coronary artery ligation was performed in a similar manner to that already described for the arrhythmias of acute coronary ischaemia, except that 30 min after coronary artery ligation a second ligature was tied tightly around the artery (Harris, 1950). The chest was subsequently closed in layers and the dog allowed to recover.

Further observations were made on the consious dog 24 hours after surgery. The dog was rested on its left side and remained in this position thereafter. Lead II of the ECG and arterial pressure (recorded by means of a cannula in the femoral artery) were recorded throughout the duration of the experiment. Having ensured that at least 80% of beats were ventricular in origin, increasing doses of drug(s) were administered through a cannula in a foreleg vein at 5 min intervals. This was continued until either sinus rhythm was restored or side effects became apparent. An example of this arrhythmia is given in figure 4.4.

Adrenaline-induced arrhythmias 3-5 days after coronary artery

ligation

Coronary artery ligation was performed in a similar manner to that already described.

Further observations were made on the conscious dog, 3-5 days after surgery. Positioning, measurement of blood pressure and electrocardiographic recording was similar to that described for the arrhythmia 24 hours after coronary artery ligation. After 10 min a dose of 10 μg/kg of adrenaline was administered intravenously and the ectopic response noted. If ventricular tachycardia or multifocal ventricular ectopic beats did not result, adrenaline administration was repeated after 10 min at a dose of 20 μg/kg. Having demonstrated an adequate arrhythmia in response to the adrenaline on 2 successive occasions, increasing doses of drug(s) were administered at 10 min intervals. 5 min after the dose of drug(s) under study, the dog was rechallenged with the test dose of adrenaline. The experiment continued until the test dose of adrenaline failed to produce any ectopic response or side effects became apparent. An example of this arrhythmia is given in figure 4.5.

With both the ouabain arrhythmia and the arrhythmia occurring 24 hours after coronary artery ligation, the ventricular rate was obtained by counting the number of sinus and ectopic beats in each successive 5 min period. For the halothane-adrenaline arrhythmia and the adrenaline-induced arrhythmia after coronary artery

ligation, the ectopic response was determined by counting the number of ventricular beats in the 5 min period following the test dose of adrenaline. In all cases, sinus beats were defined before the production of the arrhythmia. Beats with a distinct P wave preceding a mean frontal QRS vector of normal duration were defined as being of sinus origin; all others were denoted as ectopic.

4.2 Chronic Models

4.2.1 Surgical Procedure

Adult greyhounds of both sexes were anaesthetized by the intravenous administration of methohexitone sodium (10 mg/kg) and respired with 1.5% halothane and room air through an endotracheal tube using a tidal volume of 13 ml/kg and ventilation rate of 18 per min (Palmer Ideal Pump). Following thoracotomy, the fourth or fifth left rib was dissected free and removed, and the heart exposed. The anterior descending branch of the left coronary artery was dissected free below its second branch and ligated in two stages in a manner similar to that described above (section 4.1). Myocardial pacing wires (Medtronic 6400 temporary myocardial pacing leads) were sutured into the heart: one in the centre of the area supplied by the ligated artery and a second in the area jointly supplied by an adjacent branch of the anterior descending artery. Interelectrode distance was maintained at 1.0-1.5 cm in all cases. This is

illustrated in figure 4.6: the upper picture shows the exposed heart with the left anterior descending (LAD) coronary artery coursing down the left margin. In the lower picture a ligature has been passed around the origin of the apical branch of the LAD and pacing wires have been placed as described.

Both pacing wires were then brought out through skin anterior to the thoracotomy, the wound was closed in layers and the dog was allowed to recover. Routine antibiotic prophylaxis (250 mg Streptomycin and 300,000 iu Procaine Penicillin) was given intramuscularly for 3 days.

4.2.2 Protocol for Programmed Electrical Stimulation

Further observations were made on the conscious dogs, 7 to 30 days after coronary artery ligation. The dogs were positioned on their left side and remained in this position throughout the duration of the experiment. The myocardial pacing wires were attached to a Digitimer 4279 modular pacing system; leads II and III of the electrocardiogram and blood pressure (recorded by means of a cannula in the femoral artery) were continously displayed on a Devices MX4 polygraph. Drugs were administered by injection through a catheter in a foreleg vein.

Our stimulating protocol is based on that described by Lucchesi and coworkers (Wilber et al, 1985). Using bipolar pulses of 4 msec duration at twice diastolic threshold, ventricular pacing (S_1) was introduced with a basic cycle length of 350 msec (170 beats per

min). Burst pacing was then performed at rates of 200, 250, 300, 350, 400 and 450 beats per min, each for 5 seconds. If this failed to produce any arrhythmia the basic cycle length was again set at 350 msec and was not altered thereafter. An extrastimulus (S_2) was then introduced with a delay of 350 msec after the eigth basic pacing stimulus. This delay was subsequently reduced at 20 msec intervals until the extrastimulus failed to produce a ventricular response (R_2). S_2 was then set at the shortest delay which produced a ventricular response, and S_3 introduced with a delay of 350 msec after S_2. The procedure was repeated with S_3 and S_4 until an arrhythmia was produced or the the protocol was exhausted. Figure 4.7 illustrates the equipment used and practical set-up in the laboratory: the upper picture shows the post- operative dog while in the lower picture the author operates the polygraph (shown on left) and modular pacing system (shielded).

The outcome of this procedure is that the dog may be described as 'inducible', where we achieve either a sustained ventricular tachycardia (S-VT, defined as a self-perpetuating arrhythmia of more than 5 mins duration) or non-sustained ventricular tachycardia (NS-VT, defined as a reproducible arrhythmia of 4 or more ventricular ectopic beats at any given setting of $S_2/S_3/S_4$) (figure 4.8). Conversely, dogs which fail to produce S-VT or NS-VT in response to stimulation (but which exhibit some ectopic response during the stimulation protocol) are termed 'non-inducible'.

Inducible dogs were included in studies designed for the evaluation of antiarrhythmic agents (chapters 6-8), while non-inducible dogs were utilised in the evaluation of potential proarrhythmic agents (chapter 9). Dogs which failed to exhibit any ectopic response during the stimulation protocol were returned to the operating theatre for further coronary artery ligation.

In antiarrhythmic studies, drugs were administered at 5 min intervals until the arrhythmia was abolished, side effects were apparent or the dog died (ventricular fibrillation (VF) in response to stimulation). Dogs with NS-VT were rechallenged every 5 min with the stimulus setting which had produced the arrhythmia .If stimulation failed to produce an arrhythmia after drug administration, the protocol was continued as described above. Only if this failed to produce the required ectopic response was the arrhythmia considered abolished and the dog deemed non-inducible. If a dog with S-VT reverted to sinus rhythm after administration, the protocol was continued as above. Any dog still in S-VT at the end of the experiment was returned to sinus rhythm with a short burst of overdrive pacing.

In proarrhythmic studies dogs were rechallenged 5 min after each dose of drug with the stimulus setting which had produced the maximal ectopic response and this setting remained constant whether or not arrhythmias were subsequently induced. After the final dose of drug/placebo, the pacing protocol was repeated in full.

4.2.3 Electrophysiological Studies

All chronic dogs included in either antiarrhythmic or proarrhythmic drug studies with programmed electrical stimulation also had PR intervals and QRS durations measured to the nearest 5 msec (paper speed 10 cm/sec) before and after each treatment. QT intervals were similarly recorded and QT_C was calculated using the formula $QT_C=QT/\sqrt{RR}$ (Bazett,1920).

The effective refractory period (ERP) was taken as the shortest inter-stimulus interval (to the nearest 5 msec) which produced a ventricular response. Functional refractory period (FRP) was estimated from the shortest inter-response interval measured during the pacing protocol.

4.2.4 Staining of Necrotic Myocardium

All chronic dogs which died (or were destroyed) after the immediate peri-operative period had the heart removed and infarct mass determined using the method first described by Lie et al (1975).This method is based on the observation that ischaemic or infarcted myocardial cells can be distinguished from viable cells by the former's ability to reduce particular dyes to produce coloured precipitates. These dyes (in this case Triphenyl Tetrazolium Chloride, or TTC) form precipitates in the presence of intact dehydrogenase systems; necrotic areas lack such enzymatic activity and will therefore fail to stain.

The solution used in our experiments was a 1% solution of TTC

in a disodium hydrogen phosphate buffer. Since this is a light-sensitive reagent, it was made freshly when required and kept in a darkened flask.

The heart, once extracted, had all fatty tissue removed and the aortic and pulmonary roots trimmed back, before being dried and weighed (total mass). The atria were removed by cutting along the A-V grooves and the ventricles stuffed with dry tissue paper to maintain their shape. The ventricles were then placed at -20° C for 1-2 hours to firm the tissue sufficiently for accurate slicing. This was performed using a bacon slicer with the ventricular mass sectioned (10mm thickness) parallel to the A-V groove.The slices were then immersed in the TTC solution and covered with a layer of paper towel (to avoid air bubbles). Incubation proceded at 37°C for 15-20 min before the slices were rinsed in 0.9% saline and visually inspected. By this stage normal myocardium should be expected to have turned bright red and infarcted areas pale grey (figure 4.9).

After incubation, the free right ventricular wall was removed to permit calculation of left ventricular mass; the infarct (unstained tissue) was then excised and its mass expressed as a percentage of left ventricular mass.

4.3 Statistical Methods

In the ouabain-induced arrhythmia and the arrhythmia occurring 24 hours after coronary artery ligation (chapter 5), the number of ventricular ectopic beats was expressed as a fraction of

the total number of beats for two 5 min periods prior to the administration of drug. Thereafter the same parameters were counted (ventricular ectopic beats : total number of beats) for each 5 min period for the duration of the experiments. Means, standards deviations and standard errors were calculated for each group at each dose of drug, and comparisons made with the second control period (-5 min to time zero) using Student's t-tests.

With the two adrenaline-induced arrhythmias (the halothane-sensitized model and the model 3-5 days after coronary artery ligation: chapter 5), the number of ventricular ectopic beats was measured for each 5 min period following the administration of adrenaline. Means, standard deviations and standard errors were calculated for each group at each dose of drug, and comparisons made with the values for the second test dose for each group using Student's t-tests.

For the arrhythmias of acute coronary ischaemia (chapter 6), standard errors for the data obtained were sufficiently large to question a normal distribution. For this reason, the total number of ventricular ectopic beats for each of the 2 drug groups was compared with placebo using the non-parametric Mann-Whitney U test.

Results of the effects of drugs on the arrhythmias produced by programmed electrical stimulation were ranked (1,2,3) respectively for abolition of arrhythmia, no change, or death (antiarrhythmic studies, chapters 6-8) and compared with placebo using the non-

parametric Mann Whitney U test. In the proarrhythmic study (chapter 9) results of programmed electrical stimulation were ranked (0,1) for no change or induction of arrhythmia/death and compared with placebo using the Mann-Whitney U test.

In those experiments where blood pressures and heart rates were recorded, Student's t-tests were employed to compare values with pre-treatment values in the studies which did not employ placebo groups. Where placebo groups were included, variations were studied using one-way analysis of variance with the method of contrasts used to compare each group with placebo.

Variations in electrophysiological parameters (chapters 6-9) were analysed using Student's t-tests. Unless otherwise stipulated, all analyses refer to comparisons with the corresponding (pre-treatment or post-treatment) placebo value.

Inducibility of arrhythmias as a function of time after coronary artery ligation (chapter 10) was evaluated using a chi-squared contingency table analysis, the factors being time and arrhythmia status. Significant differences were taken as indicating a trend. Variations in infarct sizes as functions of both inducibility and time after coronary artery ligation were studied by one-way analysis of variance using multiple comparisons. The latter tests offer significance at the 5% level only; otherwise all statistical evaluations were performed with significance determined at the 5% and 1% levels.

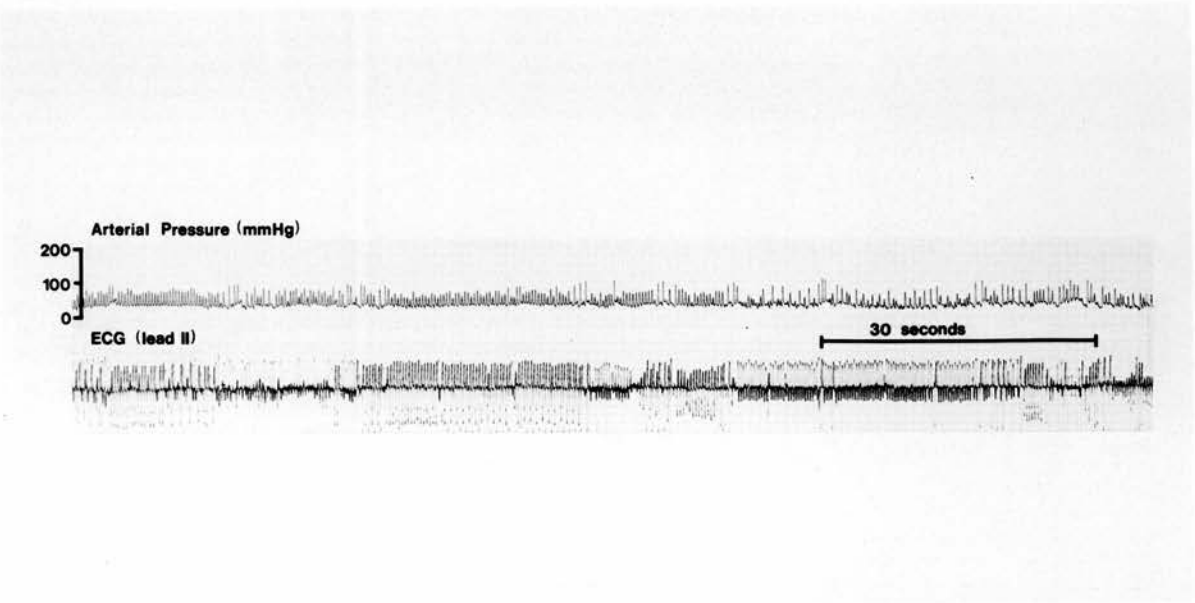
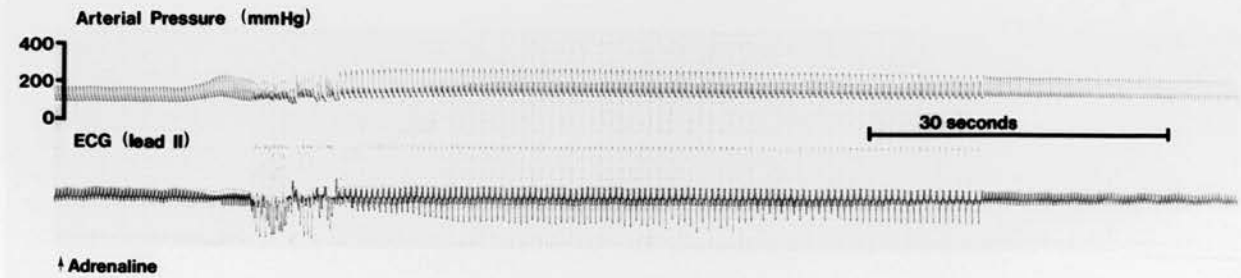


Figure 1.

Example of a ouabain-induced arrhythmia.



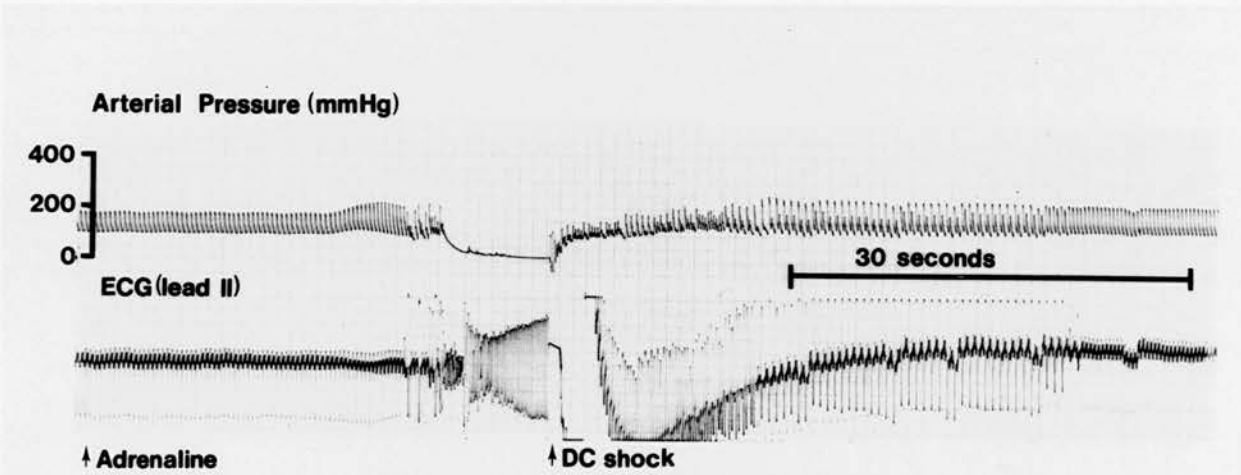


Figure 2.

Examples of the adrenaline-induced arrhythmia in a dog respired with halothane. The upper example shows a reproducible arrhythmia suitable for the testing of an antiarrhythmic compound, while the lower example shows the successful termination of ventricular fibrillation by external DC counter-shock.

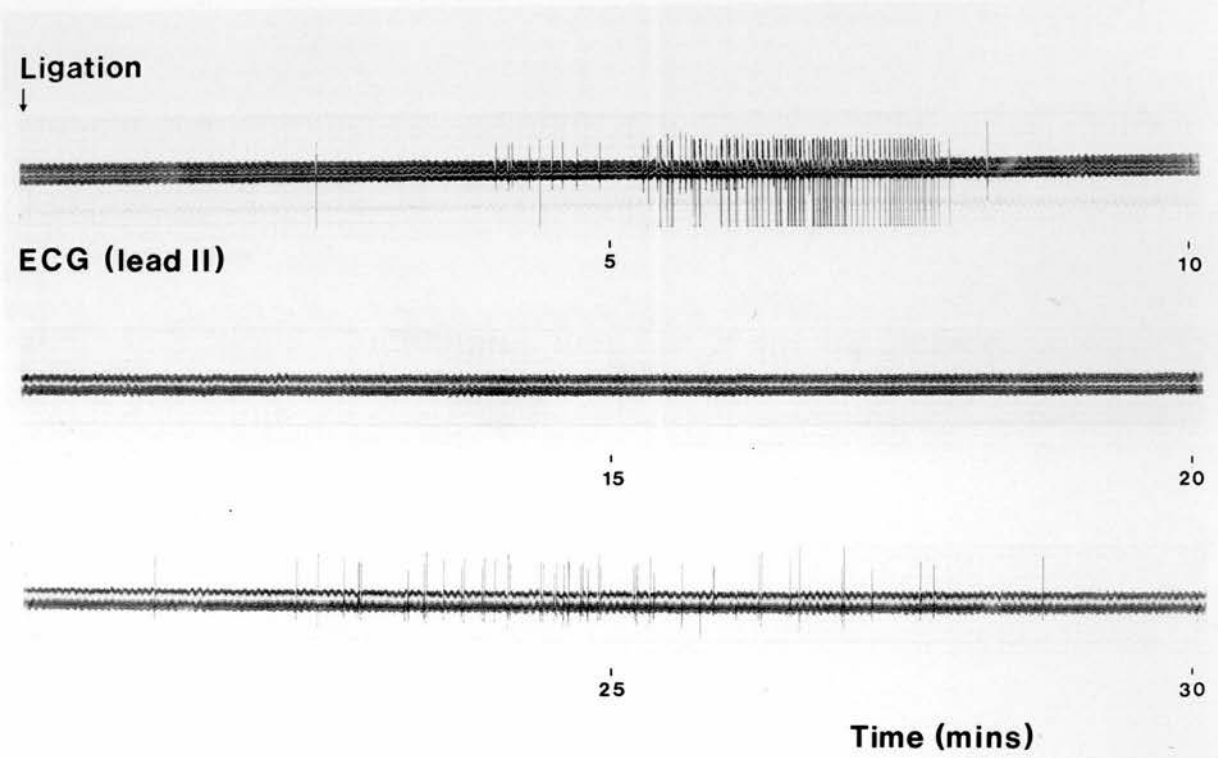


Figure 3.

Examples of the arrhythmias of acute coronary ischaemia. This continuous ECG strip shows 2 periods of arrhythmic activity: one at approximately 5-8 mins (1a) and the second at approximately 18-23 mins (1b).

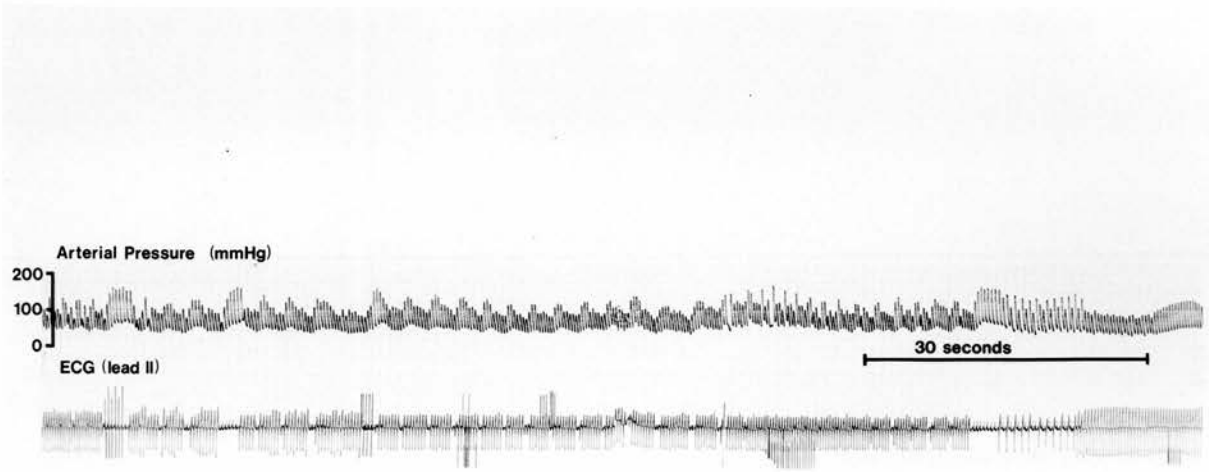


Figure 4.

Example of the arrhythmia occurring 24 hours after experimental coronary artery ligation.

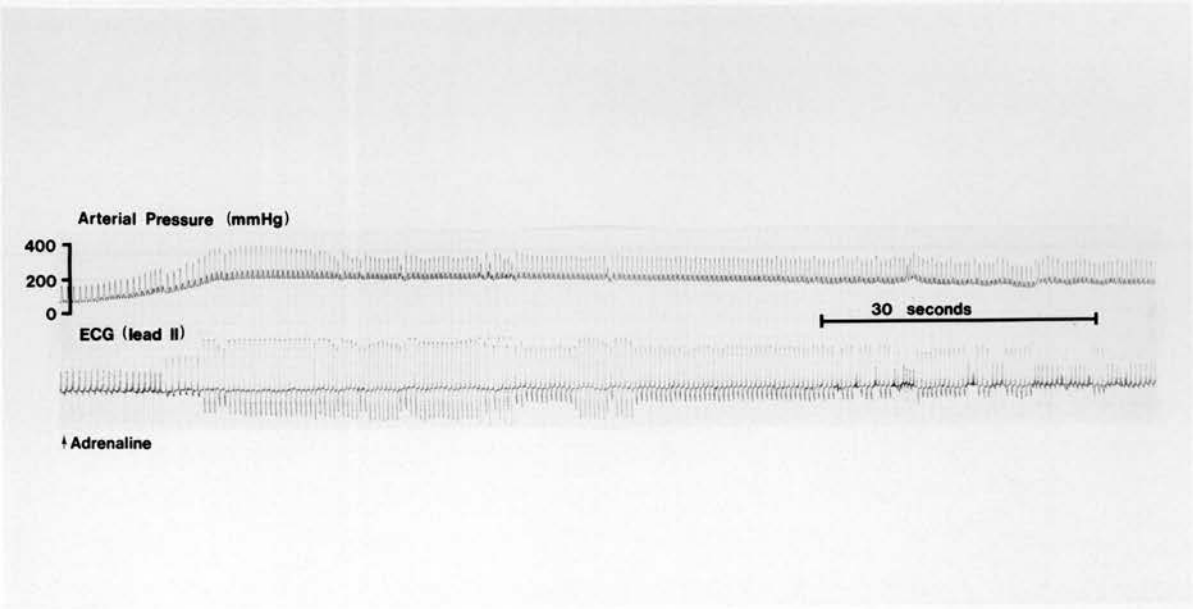


Figure 5.

Example of the adrenaline-induced arrhythmia in a conscious dog, 3-5 days after experimental coronary artery ligation.

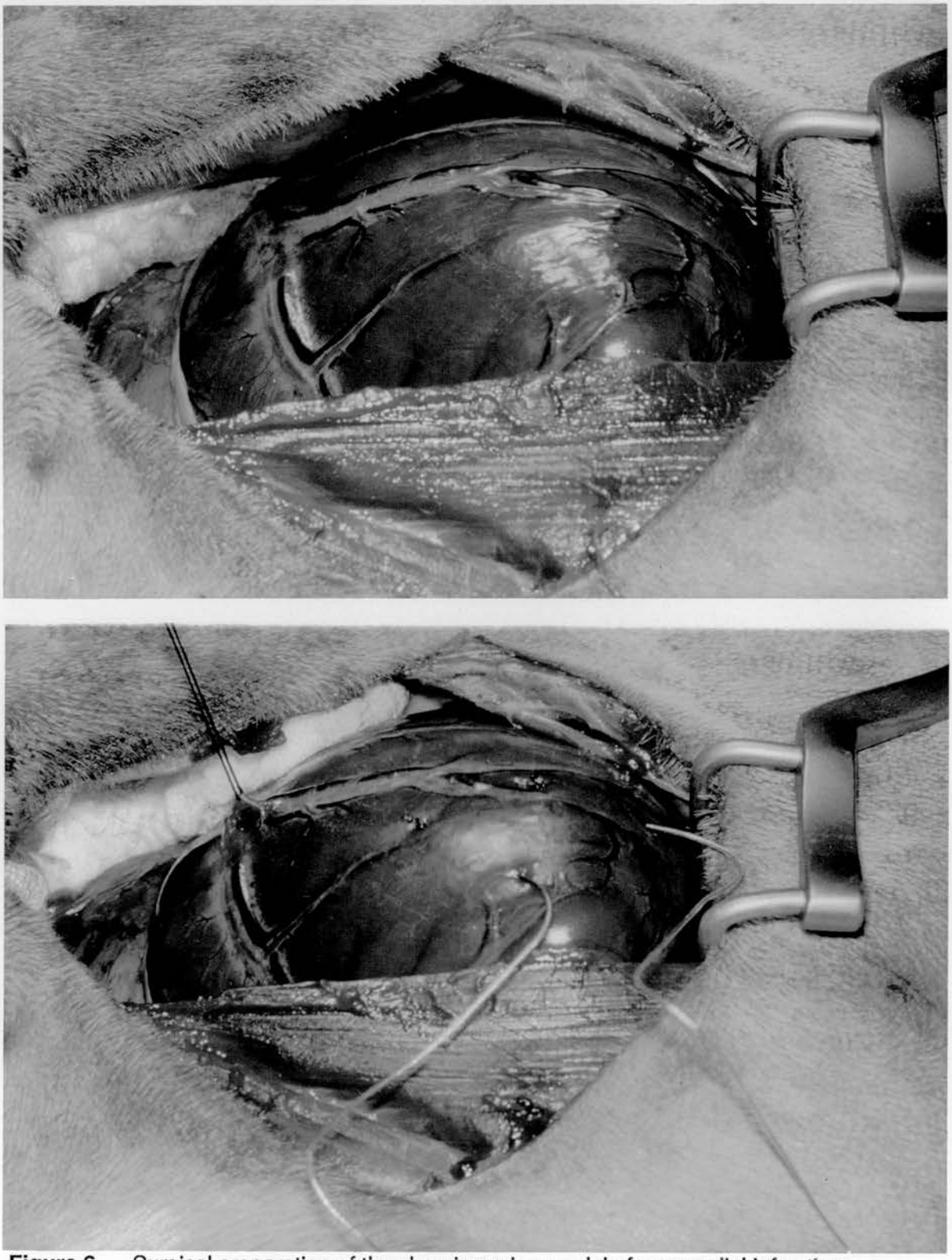
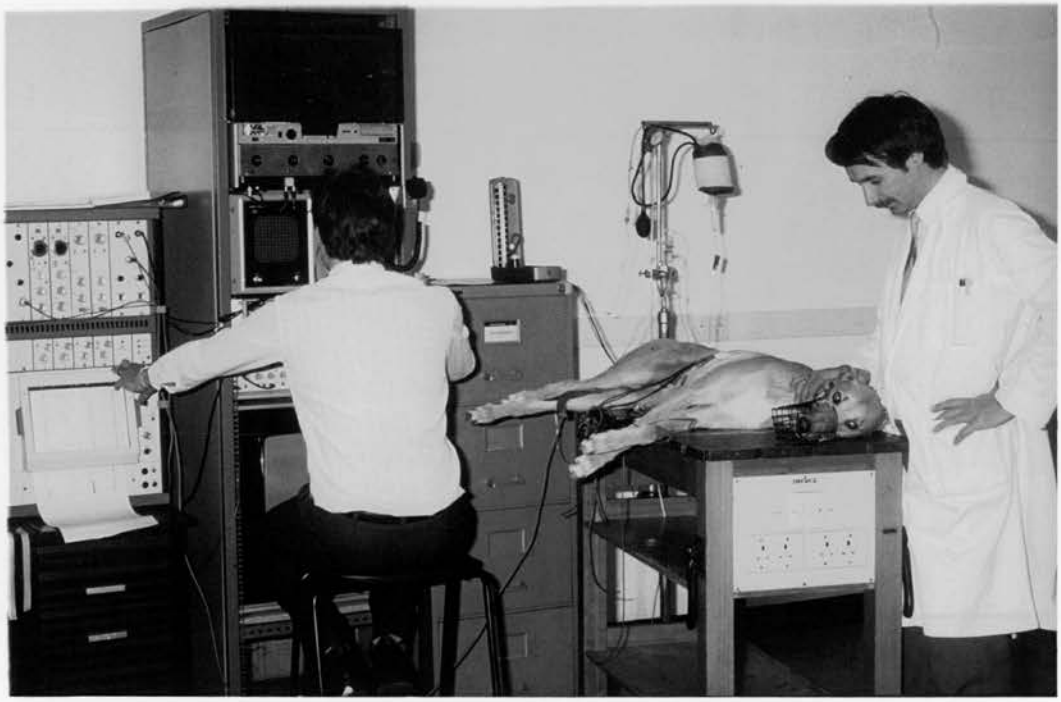


Figure 6. Surgical preparation of the chronic canine model of myocardial infarction. (See text for details).

Figure 7.
Programmed electrical Stimulation (PES)

(see text for details)



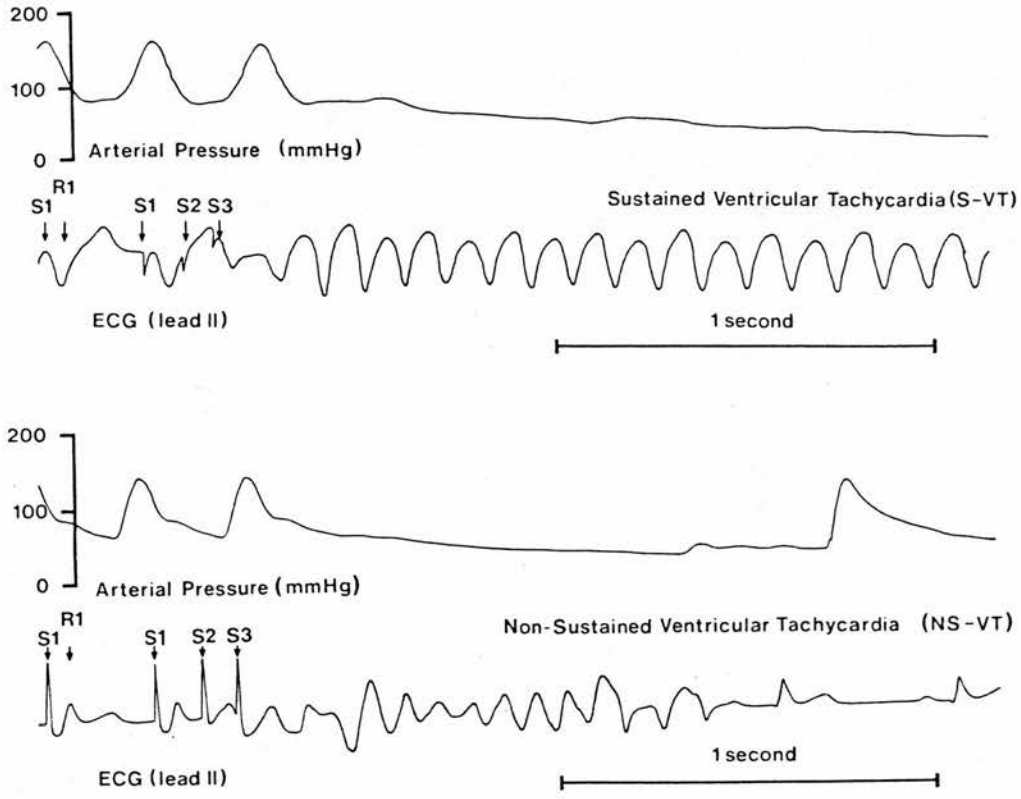


Figure 8.

Examples of sustained (upper) and non-sustained (lower) ventricular tachycardia in response to programmed electrical stimulation (PES) in conscious dogs 7-30 days after experimental coronary artery ligation.

S1,R1 Pacing stimulus and response, respectively.

S2 First extrastimulus.

S3 Second extrastimulus.

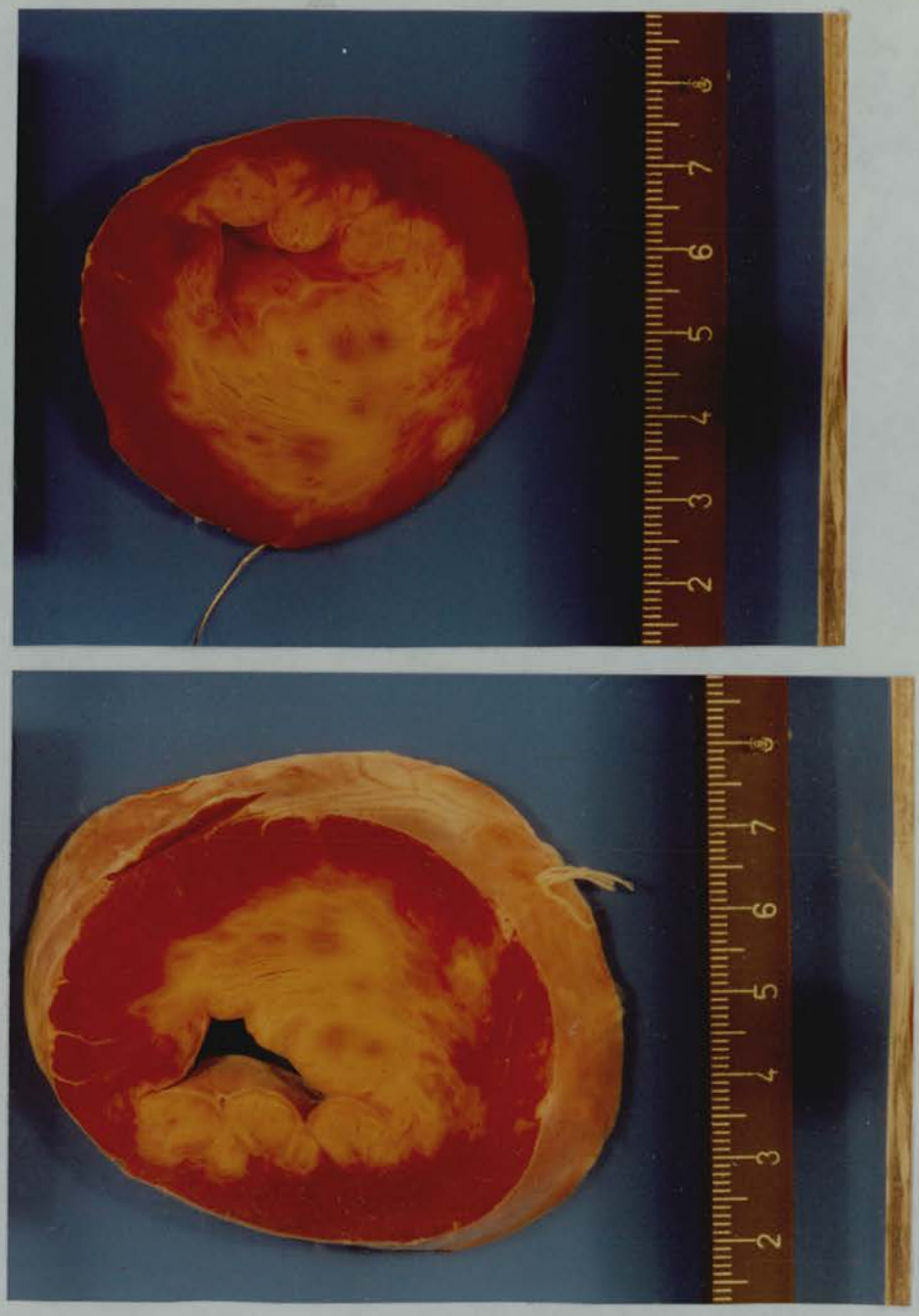


Figure 9.

Staining of necrotic myocardium using Triphenyl Tetrazolium Chloride (TTC). Normal myocardium (intact dehydrogenase systems) reduces the TTC to a bright red precipitate while infarcted areas remain pale grey.
(Note ligature in lower picture and pacing electrode on upper).

Chapter 5. EFFECTS OF THE MYOCARDIAL-SELECTIVE α-1 ADRENOCEPTOR ANTAGONIST UK-52O46 AND ATENOLOL, ALONE AND IN COMBINATION, ON EXPERIMENTAL CARDIAC ARRHYTHMIAS

Introduction

Following the introduction of the α and ß adrenergic receptor concept by Ahlquist (1948), it was generally believed that myocardial adrenergic receptors were exclusively of the ß type. It was Govier (1967, 1968) who first proposed the existence of myocardial α receptors, but despite their subsequent confirmation both in animals (Rabinowitz et al, 1975; Wagner and Brodde, 1978) and man (Schumann et al, 1978), investigation into the cardiac effects of adrenergic stimulations have continued to concentrate largely on the ß-adrenergic system. It has nevertheless been known for some time that α adrenoceptor antagonists can prevent catecholamine-induced ventricular arrhythmias (Leimdorfer, 1953). More recently it has been shown that enhanced α adrenoceptor responsiveness is a feature of myocardial ischaemia (Juhasz-Nagy and Aviado, 1976; Sheridan et al, 1980) and that this is correlated with an increase in α adrenoceptor concentration (Corr et al, 1981). Furthermore, it has been suggested that the malignant arrhythmias associated with myocardial ischaemia may be mediated primarily by α adrenoceptors (Benfey, 1982; Sheridan and Culling, 1985).

The aim of this study was to evaluate the antiarrhythmic effects of a myocardial-selective α_1 adrenoceptor antagonist UK-52046 (4-amino-6,7-dimethoxy-2-(6,7-dimethoxy-1,2,3,4-tetra hydroisoquinolin-2-yl) quinolin hydrochloride) and the $ß_1$ selective adrenoceptor antagonist atenolol, alone and in combination, in four

experimental arrhythmia models.

Methods

Four models were used in this study:

(a) The ouabain-induced arrhythmia.

(b) The halothane-adrenaline arrhythmia.

(c) The arrhythmia 24 hours after coronary artery ligation.

(d) The adrenaline-induced arrhythmia 3-5 days after coronary artery ligation.

For methodological details, please refer to chapter 4.

Drug used

Halothane (May and Baker); ± adrenaline injection B.P. (Antigen Ltd); ouabain octahydrate (Sigma Ltd); UK-52046 [4-amino-6,7-dimethoxy-2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl) quinolin hydrochloride] was available as the methane sulphonate, MW 490 (Pfizer Laboratories Ltd); atenolol (Stuart Pharmaceuticals Ltd). Dose of UK-52046 and atenolol are expressed in terms of the free base.

Statistics

Details covered in chapter 4.

Results

Ouabain-induced arrhythmias

Observations were made in 2 groups of 6 dogs in which the intravenous administration of ouabain had produced a rapid multifocal ventricular tachycardia. The required doses of ouabain (65.8 ± 7.4 (mean ± SEM) μg/kg and 68.3 ± 4.2 μg/kg for UK-52046 and atenolol groups respectively) were similar to those in a previous study which established that this arrhythmia persists for more than 2 hours (Allen et al, 1971). After the arrhythmia had been established for 10 min, drugs were given intravenously in increasing doses at intervals of 5 min.

The administration of up to 64 μg/kg UK-52046 had no effect upon the number of sinus beats in each 5 min period (figure 5.1). mean systolic pressure fell from an initial 295.0 ± 27.0 to 198.0 ± 31.3 mmHg after 2.0 μg/kg ($P<0.05$). After 32 μg/kg of UK-52046, this had further fallen to 110.0 ± 9.1 mmHg ($P<0.01$).

The effects of atenolol are summarised in figure 5.2. The administration of up to 400 μg/kg of atenolol had no effect upon the number of sinus beats in each 5 min period; after 800 μg/kg the number of sinus beats fell ($p<0.05$). Mean systolic pressure fell from an initial 220.0 ± 14.8 to 193.3 ± 18.7 after 12.5 μg/kg of atenolol ($p<0.05$). After 800 μg/kg this had further fallen to 140.0 ± 25 mmHg ($p<0.01$).

Halothane-adrenaline arrhythmias

Observations were made in 3 groups of 6 anaesthetised dogs. The mean dose of adrenaline required to produce an arrhythmia in

each group was 4.3 ± 0.7 μg/kg (UK-52046), 4.0 ± 0.9 μg/kg (atenolol) and 7.6 ± 0.7 μg/kg (combination).

In the UK-52046 group, the mean number of ectopic beats after the test dose of adrenaline was 52.2 ± 6.6 μg/kg. After 0.25 μg/kg of drug this had fallen to 28.8 ± 7.7 ($p<0.05$). The arrhythmia was prevented by a mean dose of 3.8 ± 1.4 μg/kg of UK-52046 (figure 5.3).

The test dose of adrenaline produced a significant increase in blood pressure before the onset of arrhythmia, with the peak blood pressure response occurring shortly after the start of arrhythmia. With doses of UK-52046 which prevented the arrhythmia, this pressor response to adrenaline was significantly reduced (table 5.1); Initial blood pressure (before the administration of adrenaline) was also reduced compared with control values (169.2 ± 28.1 and 216.7 ± 29.2 mmHg respectively) but the difference did not reach statistical significance. No change in heart rate was observed as the result of UK-52046 in doses of up to 8 μg/kg (figure 5.3).

In the atenolol group the mean number of ectopic beats after the test dose of adrenaline was 32.0 ± 5.2. This had fallen ($p<0.01$) after 6.25 μg/kg of drug and the arrhythmia was prevented by a mean dose of 14.6 ± 2.1 μg/kg of atenolol (figure 5.4). Doses of atenolol which prevented the arrhythmia were associated with a fall in blood pressure, but this was not significant and the pressor response to adrenaline was maintained (table 5.1). Atenolol at doses of 12.5 μg/kg and 25 μg/kg produced significant reductions in heart

rate compared with placebo (147.0 ± 4.1, 142.0 and 168.8 ± 7.5 per min respectively), but the adrenaline-induced increase in heart rate seen in the control arrhythmia was maintained in the protected arrhythmia (table 5.1).

In the group which were given a combination containing equal amounts of UK-52046 and atenolol the mean number of ectopic beats in the control arrhythmia was 97.8 ± 17.1. This had fallen ($p<0.05$) after 0.25 μg/kg of each drug and was prevented after a mean dose of 0.36 ± 0.1 μg/kg of each (figure 5.5). The pressor response to adrenaline was maintained at doses which prevented the arrhythmia. The combination in doses of up to 1 μg/kg of each drug had no effect on heart rate but the adrenaline-induced increase in heart rate was attenuated in the protected arrhythmias (table 5.1).

Arrhythmias 24 hours after coronary artery ligation

Observations were made in 2 groups of 6 conscious dogs, 22-24 hours after experimental coronary artery ligation. The number of sinus beats varied among dogs during the control period but was never greater than 20%.

The effects of UK-52046 are summarised in figure 5.6. After 16 μg/kg and 32 μg/kg of drug the number of sinus beats in each 5 min period had increased from a control value of 137.7 ± 47 μg/kg to 586 ± 128.9 μg/kg and 662.3 ± 99.1 μg/kg respectively ($p<0.01$). These 2 doses of UK-52046 were also associated with a fall ($p<0.05$) in the total ventricular rate. Blood pressure fell ($p<0.05$) with doses

	BEFORE ADRENALINE	AT PEAK RESPONSE	AT ONSET OF ARRHYTHMIA
DRUG	MEAN SYSTOLIC PRESSURE (mmHg)		
CONTROL ARRHYTHMIA			
UK-52046	216.7 ± 29.2	**304.2 ± 34.9	**242.5 ± 30.2
Atenolol	211.7 ± 21.1	**288.3 ± 19.1	**255.8 ± 16.0
Combination	145.8 ± 18.0	**318.3 ± 24.1	**235.8 ± 29.4
PROTECTED ARRHYTHMIA			
UK-52046	169.2 ± 28.1	177.5 ± 27.4	
Atenolol	157.5 ± 14.2	**200.0 ± 12.9	
Combination	135.0 ± 18.4	**245.0 ± 14.3	
	HEART RATE (beats/min)		
CONTROL ARRHYTHMIA			
UK-52046	171.0 ± 9.2	178.7 ± 4.2	187.3 ± 6.4
Atenolol	168.8 ± 7.5	*183.0 ± 8.2	*183.0 ± 7.2
Combination	153.3 ± 6.8	*199.0 ± 5.5	*191.7 ± 5.2
PROTECTED ARRHYTHMIA			
UK-52046	168.3 ± 10.0	172.0 ± 6.7	
Atenolol	145.0 ± 3.9	*154.0 ± 3.7	
Combination	150.3 ± 10.8	173.0 ± 8.4	

Table 5.1

Arterial pressure and heart rate (mean ± SEM) during the halothane-adrenaline arrhythmia recorded before the test dose of adrenaline, at peak response and at the onset of the arrhythmia. Values are given for responses before the various treatments (control arrhythmia) and after preventative doses of the respective therapies (protected arrhythmia).

*$p<0.05$ **$p<0.01$ (compared with values before adrenaline).

of 8 μg/kg and above.

Atenolol in doses of up to 800 μg/kg had no effect upon the same arrhythmia in a second group of 6 conscious dogs (figure 5.7). Blood pressure did not vary during the course of the experiment.

Adrenaline-induced arrhythmia 3-5 days after coronary artery ligation

Observations were made in 3 groups of 6 conscious dogs. The dogs were studied 72 hours after coronary artery ligation but only included if an initial 15 min ECG recording showed no evidence of arrhythmia. If any ectopics were observed the experiment was delayed a further 24 hours. Any dog not in sinus rhythm at 120 hours was excluded from the study. The mean dose of adrenaline required to produce an arrhythmia in each group was 15.0 ± 2.2 μg/kg (UK-52046), 15.0 ± 2.2 μg/kg (atenolol) and 18.3 ± 4.0 μg/kg (combination).

In the UK-52046 group the mean number of ectopic beats after the test dose of adrenaline was 265.7 ± 33.4 μg/kg; a significant reduction was apparent after 0.25 μg/kg of UK-52046 (128.0 ± 24.5 μg/kg, $p<0.01$) and the arrhythmia was prevented in 6/6 dogs with a mean dose of 3.7 ± 1.4 μg/kg (figure 5.8). There was no significant change in blood pressure although the pressor response to adrenaline was reduced from 153.7 ± 26.5 mmHg in the control arrhythmia to 58.7 ± 4.3 mmHg in the protected state ($p<0.05$). Compared with the control period, heart rate increased ($p<0.01$) after 1 μg/kg of drug

(117.7 ± 10.4 and 138.3 ± 10.6 beats per min respectively). After 8 μg/kg this had further increased to 157.0 ± 7.0 per min.

The results for the atenolol group are summarised in figure 5.9. The mean number of ectopic beats after adrenaline was 277.5 ± 91.2; after 100 μg/kg atenolol this had fallen to 31.1 ± 17.2 representing an 84.4% reduction ($p<0.01$). Further increases in the dose of atenolol were not possible because of increasing agitation in the dogs. There was no change in blood pressure, and the pressor response to adrenaline although attenuated compared with control values, was still highly significant (140 ± 11.9 mmHg and 173 ± 13.5 mmHg respectively).

In the group studied with a combination of UK-52046 and atenolol, the mean number of ectopic beats after adrenaline was 140.5 ± 31.5. The arrhythmia was prevented in 6/6 dogs after a mean dose of 3.7 ± 1.1 μg/kg of each drug. With the arrhythmia protected the pressor response to adrenaline was not significantly reduced and overall blood pressure did not change. However, as with the UK-52046 group, heart rate increased ($p<0.01$) after 2 μg/kg reaching a maximum of 146.3 ± 6.8 beats/min after the final dose ($p<0.01$ compared with control value of 104.8 ± 8.7 per min) (figure 5.10).

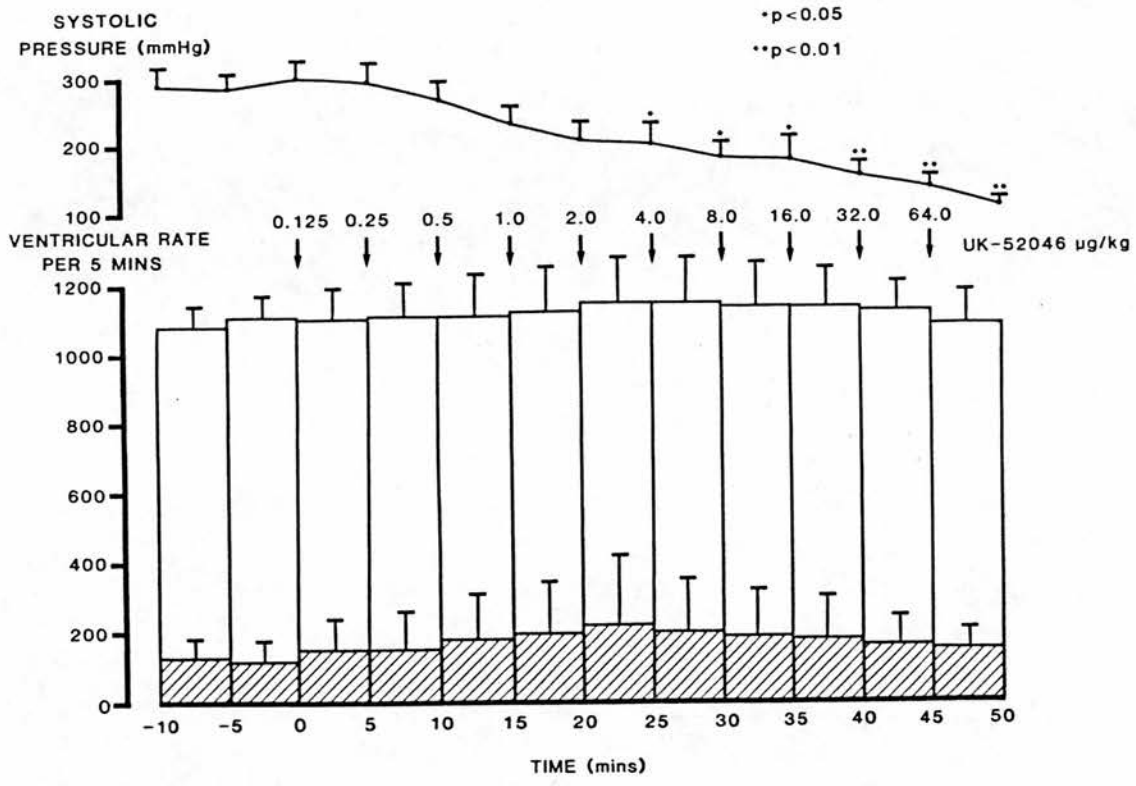


Figure 1.

Effects of the intravenous administration of UK-52046 on the ouabain-induced arrhythmia in a group of 6 anaesthetized dogs. The histograms show the ventricular rate (clear columns) and number of sinus beats (shaded) for each 5 min period. Values are expressed as mean±SEM.

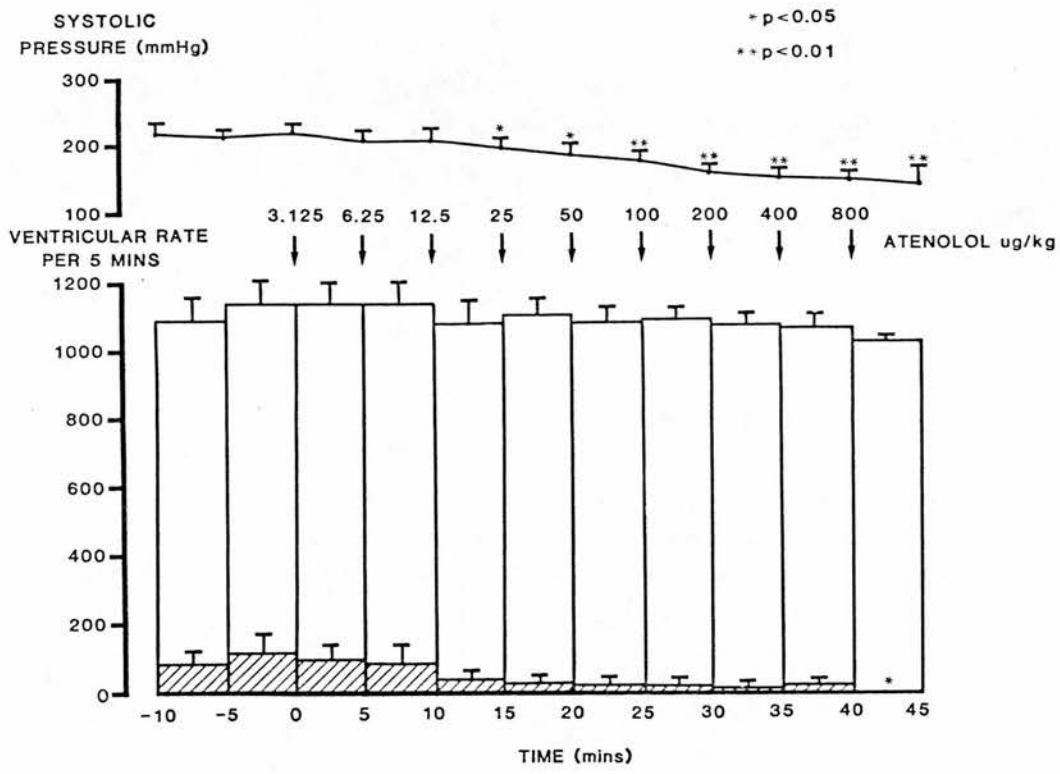


Figure 2.

Effects of the intravenous administration of atenolol on the ouabain-induced arrhythmia in a group of 6 anaesthetized dogs. The histograms show the ventricular rate (clear columns) and number of sinus beats (shaded) for each 5 min period. Values are expressed as mean±SEM.

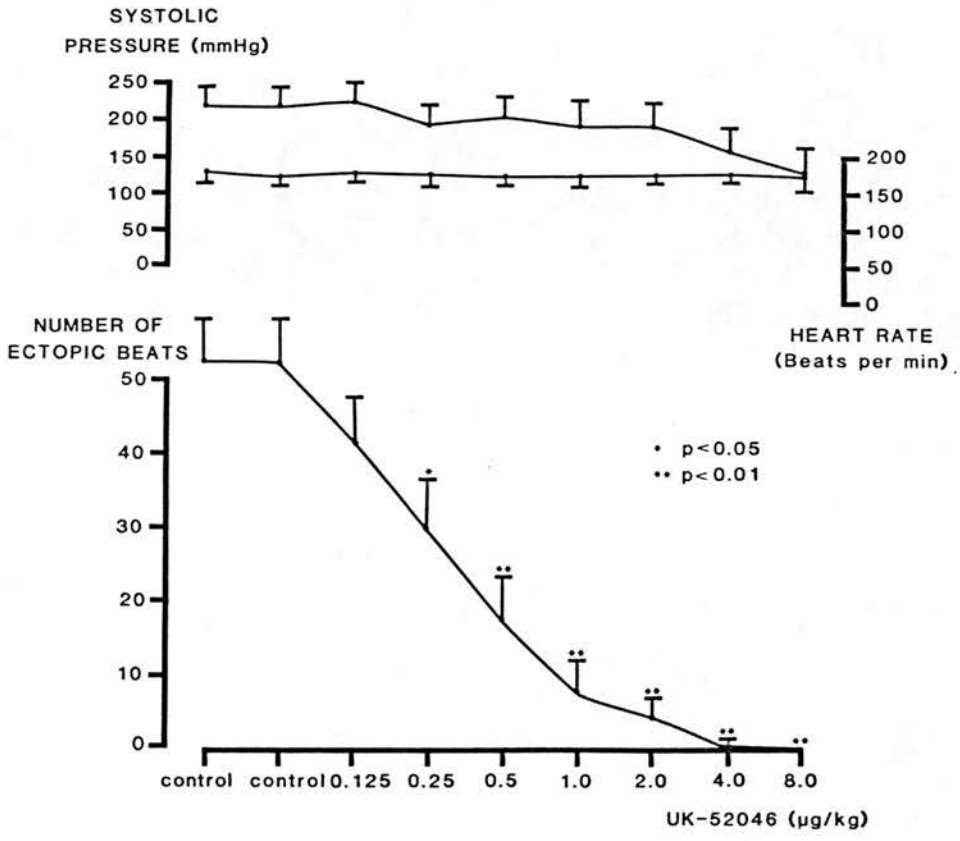


Figure 3.

Effects of the intravenous administration of UK-52046 on the halothane-adrenaline arrhythmia in a group of 6 anaesthetized dogs. Dose of adrenaline required to produce the arrhythmia was 4.3±0.7 ug/kg. Values are expressed as mean±SEM.

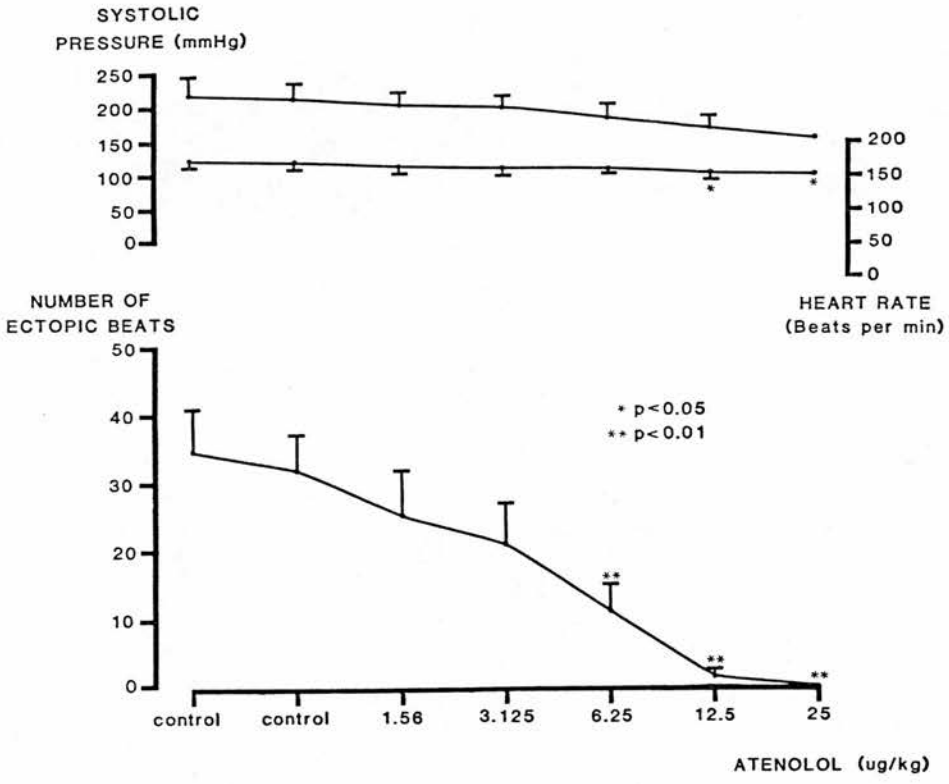


Figure 4.

Effects of the intravenous administration of atenolol on the halothane-adrenaline arrhythmia in a group of 6 anaesthetized dogs. Dose of adrenaline required to produce the arrhythmia was 4.0±0.9 ug/kg. Values are expressed as mean±SEM.

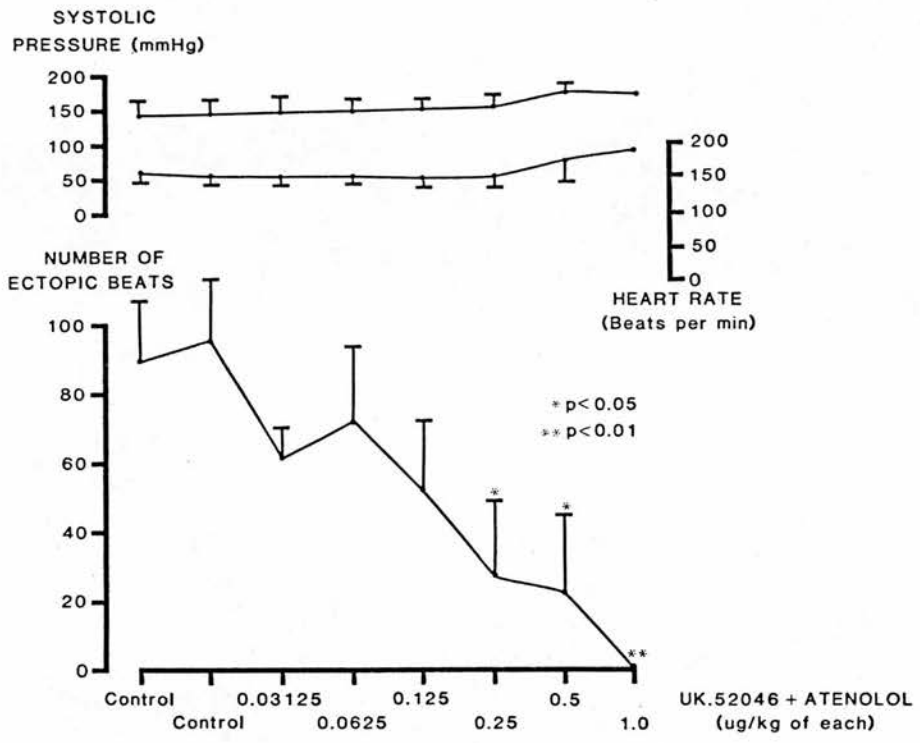


Figure 5.

Effects of the intravenous administration of a combination containing equal parts of UK-52046 and atenolol on the halothane-adrenaline arrhythmia in a group of 6 anaesthetized dogs. Dose of adrenaline required to produce the arrhythmia was 7.6±0.7 ug/kg. Values are expressed as mean±SEM.

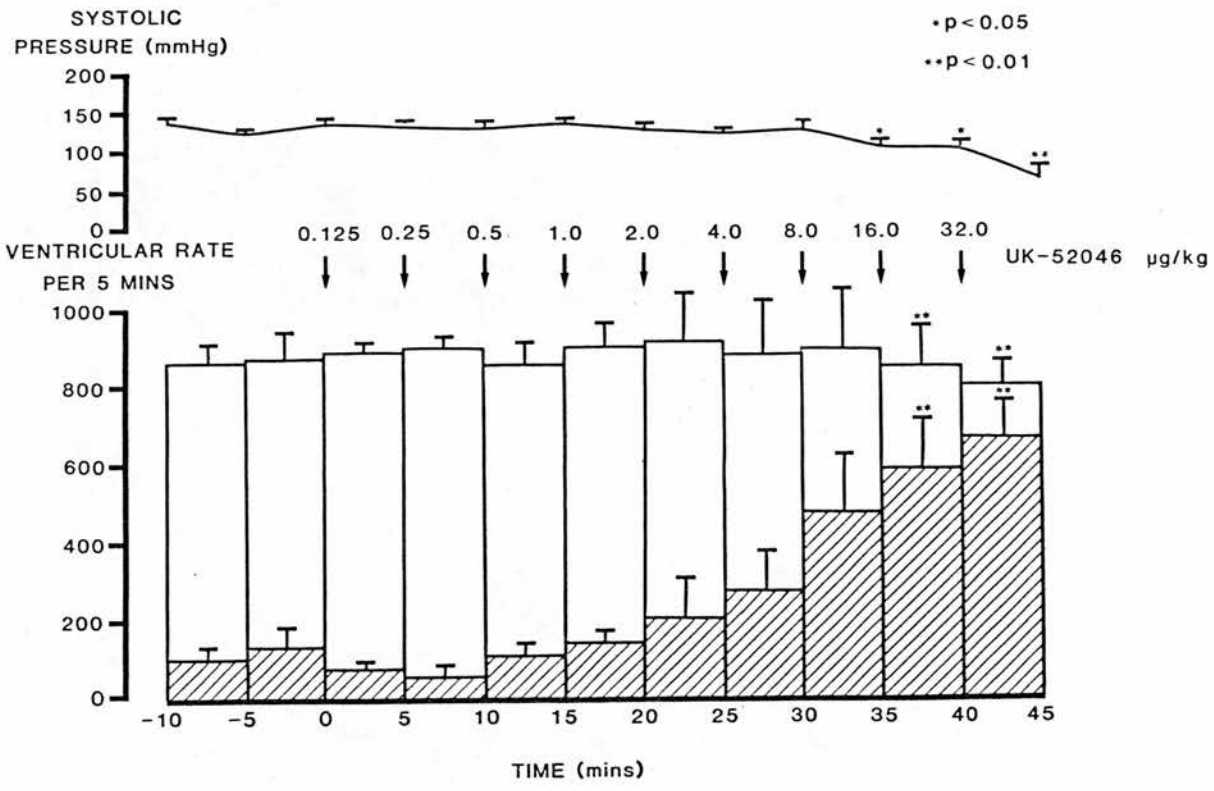


Figure 6.

Effects of the intravenous administration of UK-52046 upon the ventricular arrhythmia occurring 24 hours after experimental coronary artery ligation. The histograms show the ventricular rate (clear columns) and number of sinus beats (shaded) for each 5 min period. Values are expressed as mean ±SEM.

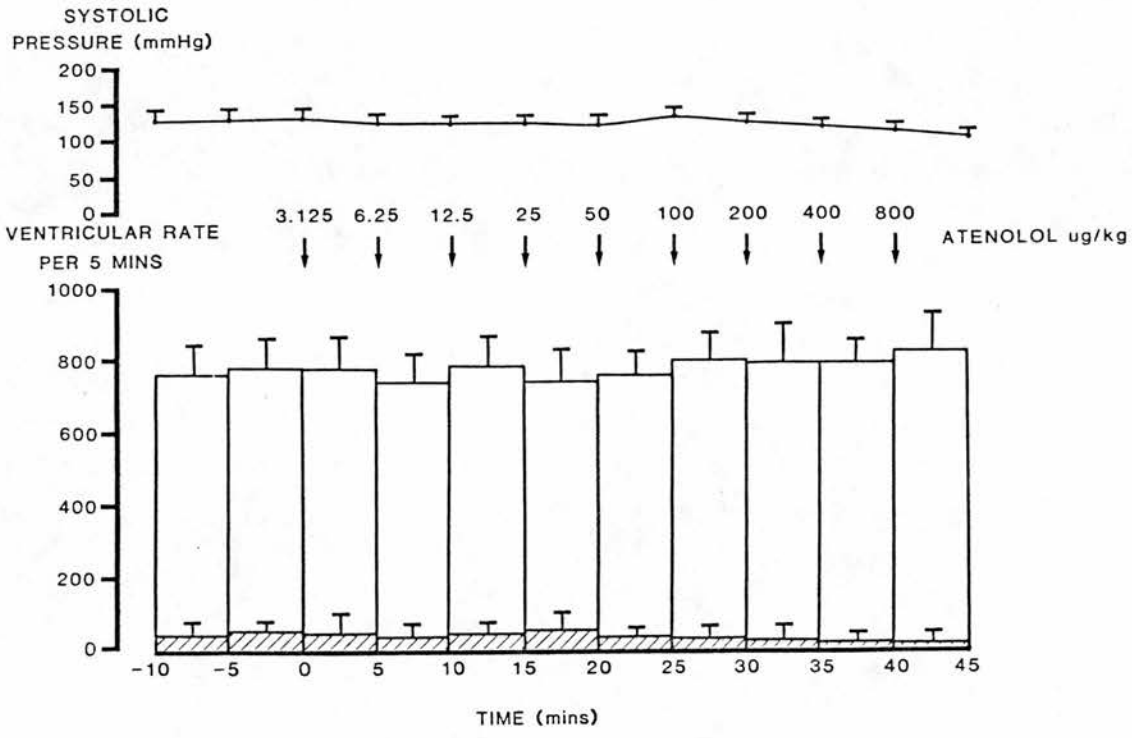


Figure 7.

Effects of the intravenous administration of atenolol upon the ventricular arrhythmia occurring 24 hours after experimental coronary artery ligation. The histograms show the ventricular rate (clear columns) and number of sinus beats (shaded) for each 5 min period. Values are expressed as mean ±SEM.

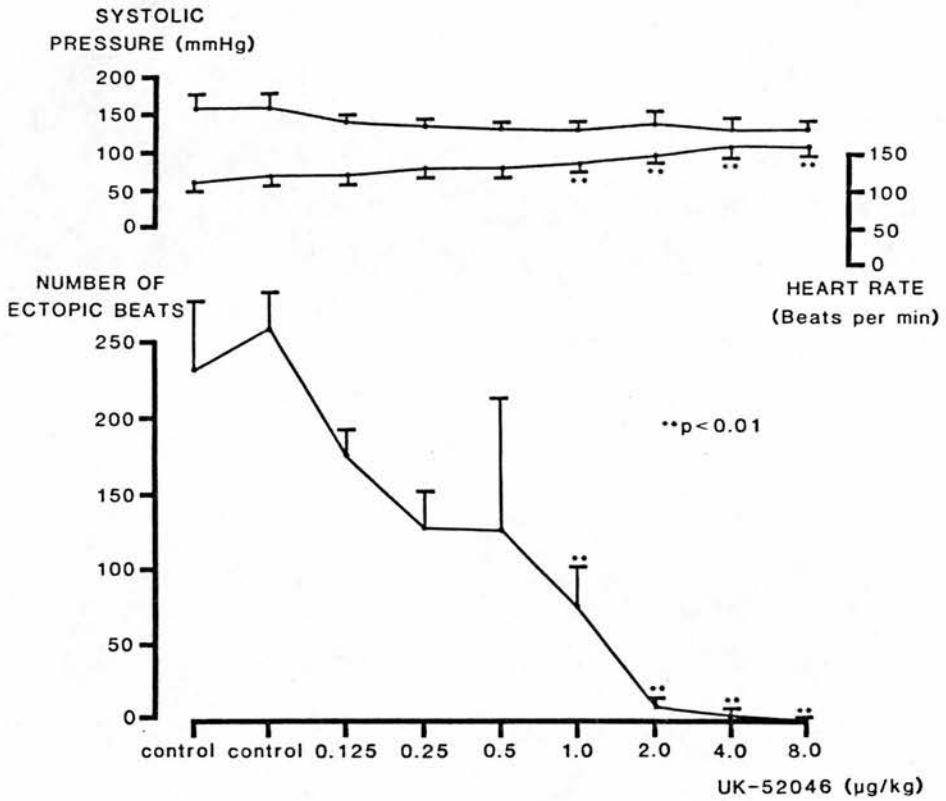


Figure 8.

Effects of the intravenous administration of UK-52046 upon the adrenaline-induced arrhythmia in a group of 6 conscious dogs, 3-5 days after experimental coronary artery ligation. Dose of adrenaline required to produce the arrhythmia was 15.0±2.2 ug/kg (mean±SEM).

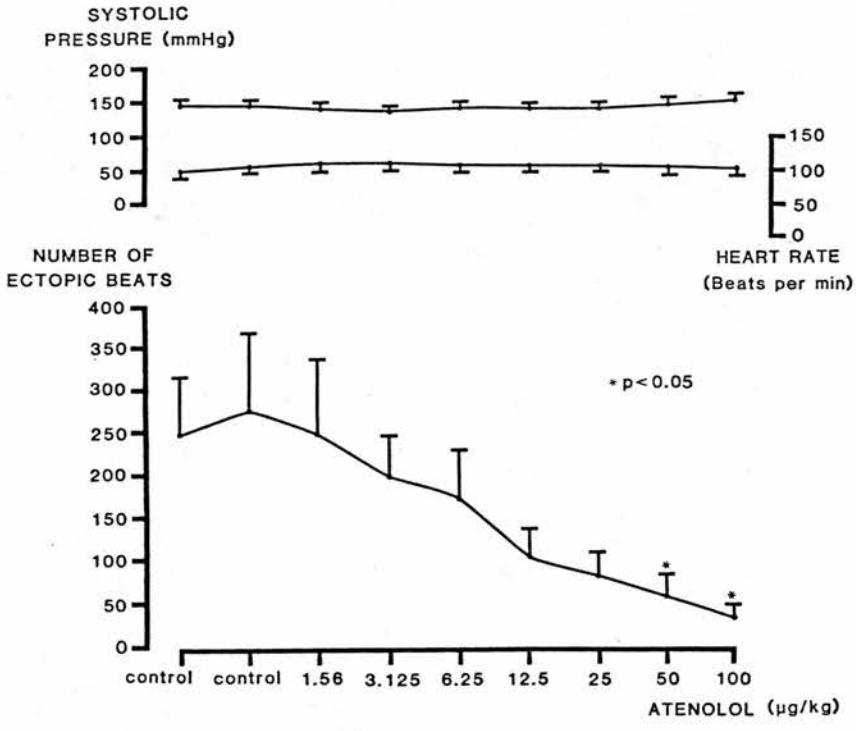


Figure 9.

Effects of the intravenous administration of atenolol upon the adrenaline-induced arrhythmia in a group of 6 conscious dogs, 3-5 days after experimental coronary artery ligation. Dose of adrenaline required to produce the arrhythmia was 15.0±2.2 ug/kg (mean±SEM).

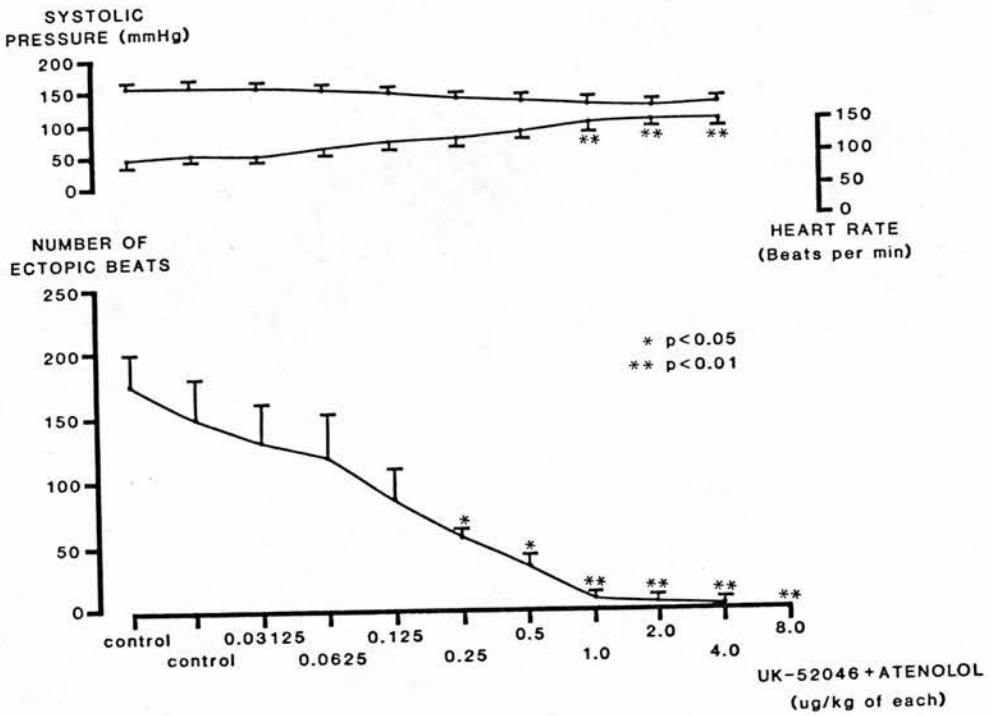


Figure 10.

Effects of the intravenous administration of a combination containing equal parts of UK52046 and atenolol upon the adrenaline-induced arrhythmia in a group of 6 conscious dogs, 3-5 days after experimental coronary artery ligation. Dose of adrenaline required to produce the arrhythmia was 7.6±0.7 ug/kg (mean±SEM).

Chapter 6. EFFECTS OF THE MYOCARDIAL-SELECTIVE α-1 ADRENOCEPTOR ANTAGONIST UK-52046 ON TWO EXPERIMENTAL REENTRANT ARRHYTHMIAS

Introduction

Following the beneficial reports of UK-52046 as an antiarrythmic agent in various experimental models where arrhythmia production is dependent upon enhanced automaticity (chapter 5) it was decided to extend studies with the drug to include two models which depend upon reentrant mechanisms for arrhythmia genesis.

Methods

The models used in this study included the arrhythmias of acute coronary ischaemia and the arrhythmias produced by programmed electrical stimulation in a chronic canine model of myocardial infarction. The latter model also included the measurement of electrocardiographic parameters and refractoriness.

For methodological details, please refer to chapter 4.

Drugs Used

UK-52046 (4-amino-6,7-dimethoxy-2-(6,7-dimethoxy-1,2,3, 4-tetrahydroisoquinolin-2-yl) Quinolin hydrochloride). The drug was available as the methane sulphonate, molecular weight 490 (Pfizer Laborotories Ltd). All doses are expressed in terms of the free base.

Statistics

Details covered in chapter 4.

Results

Arrhythmias following acute myocardial ischaemia

A total of 24 dogs were studied. The median number of ventricular ectopic beats in the 12 dogs which received placebo was 190 (range 4-674), with peaks at 5-10 min and 20-25 min (values given as mean ± SEM in figure 6.1, table 6.1). The corresponding value for the group which received UK-52046, 4 μg/kg was 246 (9-1204), again with a biphasic distribution (table 6.1); In the group which received UK-52046, 8 μg/kg, the number of ectopic beats was significantly reduced with a median of 12 (range 1-154).

Mean initial systolic blood pressures for the 3 groups were respectively 75.9 ± 8.3 mmHg (placebo), 74.2 ± 12.2 mmHg (UK-52046, 4 μg/kg) and 80.8 ± 6.7 mmHg (UK-52046, 8 μg/kg) (differences not statistically significant). However, both 4 μg/kg and 8 μg/kg of drug produced significant falls in systolic pressure which persisted throughout the duration of the experiments (table 6.1).

Programmed Electrical Stimulation

Two groups of six inducible dogs were randomly allocated to receive increasing intravenous doses of UK-52046 or placebo.

For the 6 dogs in the placebo group, programmed stimulation resulted in a reproducible NS-VT in 5 dogs and a sustained VT in one dog prior to treatment. Of the 5 dogs with NS-VT, 3 remained

inducible when stimulated after each of 7 doses of placebo; the other 2 developed VF when stimulated 5 min after the first dose of placebo. The dog with S-VT remained in this arrhythmia throughout the duration of the experiment (figure 6.2).

The effects of UK-52046 on the arrhythmias produced by PES are illustrated in figure 6.3. In this group 5/6 dogs had a NS-VT and one was in S-VT prior to treatment. Of the 5 with NS-VT, 2 became non-inducible after 4.0 μg/kg of drug, one remained unchanged and 2 died when stimulated after 8 μg/kg and 64 μg/kg respectively. The dog with S-VT remained in this arrhythmia throughout the duration of the experiment. UK-52046 was well tolerated up to doses of 64 μg/kg. Statistical analysis indicated that, when compared with placebo, UK-52046 did not exhibit significant antiarrhythmic activity.

Blood Pressure and Heart Rate

In the placebo group mean initial systolic pressure for the 6 dogs was 155 ± 6.9 (mean ± SEM) mmHg. The corresponding heart rate was 114.2 ± 12.0 per min. Neither value altered significantly during the experiment (figure 6.2).

Mean intial systolic pressure for the group receiving UK-52046 was 171 ± 5.6 mmHg. After 4 μg/kg this had fallen to 136 ± 13.6 mmHg ($p<0.05$ compared with placebo); further falls were apparent with increasing doses of drug (figure 6.3). Heart rate rose from an initial 95.8 ± 7.5 per min to 116.7 ± 5.9 per min (difference

not statistically significant). Pre-treatment figures for the placebo group were not statistically different from the corresponding values for the 6 dogs receiving UK-52046, nor was any change observed as a result of drug therapy (table 6.2). pre-treatment figures for the placebo group were not statistically different from the corresponding values for the 6 dogs receiving UK-52046, nor was any change observed as a result of drug therapy (table 6.2).

Electrophysiological Measurements

The effects of placebo and UK-52046 upon the PR interval, QRS duration, QT_c and refractory periods are summarised in table 6.2. Mean PR for the 6 dogs before placebo was 0.11 sec; after placebo this had increased to 0.115 sec, but the difference was not statistically significant. Similarly, no charge occured in mean QRS (0.08-0.085), mean QT_c (0.30-0.31 sec), mean ERP (0.105-0.11 sec) and mean FRP (0.165-0.17 sec) before and after placebo respectively. The pre-treatment figures for the placebo group were not statistically different from the corresponding values for the 6 dogs receiving UK-52046, nor was any charge observed as the result of drug therapy (table 6.2)

NUMBER OF ECTOPIC BEATS

DRUG	n	Time following coronary artery ligation (min) '0-5	5-10	10-15	15-20	20-25	25-30	total no
PLACEBO	12	14.7 ±8.8	47.2 ±31.5	12.1 ±8.3	25.7 ±18.8	101.2 ±37.7	56.2 ±13.9	190 (4-674)
UK-52046 4 µg/kg	6	3.1 ±1.9	41.3 ±38.9	5.5 ±5.1	1.8 ±1.8	244.6 ±131.2	123.2 ±59.8	246 (9-1204)
UK-52046 8 µg/kg	6	5.8 ±5.8	6.3 ±5.2	7.6 ±5.7	9.6 ±7.2	7.5 ±6.3	4.8 ±3.0	12 (1-154)

SYSTOLIC BLOOD PRESSURE

DRUG	n	Time following coronary artery ligation (min) Before drug	0	5	10	15	20	25	30
PLACEBO	12	75.9 ±8.3	82.1 ±7.7	90.0 ±7.9	91.7 ±6.6	90.4 ±6.2	91.8 ±5.4	90.0 ±4.9	88.8 ±4.9
UK-52046 4 µg/kg	6	74.2 ±12.2	65.8 ±6.6	64.2* ±6.4	65.0* ±7.4	67.5* ±7.4	65.8* ±6.5	64.0* ±8.0	69.1 ±8.3
UK-52046 8 µg/kg	6	80.0 ±6.8	54.2* ±7.5	52.5** ±6.7	50.0** ±5.0	57.5** ±12.7	58.5* ±12.1	65.0* ±10.7	64.0* ±10.4

Table 6.1

Number of ventricular ectopic beats (upper) and mean systolic blood pressures (lower) in response to acute coronary ischaemia in 3 groups of anaesthetized dogs. Coronary artery ligation was performed 5 min after administration of placebo (n=12), UK-52046 4µg/kg (n=6) or UK-52046 8µg/kg (n=6) *$p<0.05$ **$p<0.01$ (compared with placebo).

Placebo

Parameter	Before Drug	After Drug
QT_C	0.30 ± 0.01	0.31 ± 0.02
QRS	0.08 ± 0.005	0.085 ± 0.005
PR	0.011 ± 0.005	0.115 ± 0.005
ERP	0.105 ± 0.005	0.11 ± 0.005
FRP	0.105 ± 0.005	0.11 ± 0.01

UK-52046

Parameter	Before Drug	After Drug
QT_C	0.26 ± 0.02	0.295 ± 0.01
QRS	0.07 ± 0.005	0.075 ± 0.005
PR	0.011 ± 0.005	0.10 ± 0.005
ERP	0.125 ± 0.005	0.12 ± 0.005
FRP	0.18 ± 0.005	0.175 ± 0.01

Table 6.2

Effects of placebo and UK-52046 on the corrected QT internal (QT_C), QRS duration (QRS), PR interval (PR) and effective (ERP) and functional (FRP) refractory periods in 2 groups of 6 dogs.
Results are expressed in seconds (mean ± SEM).

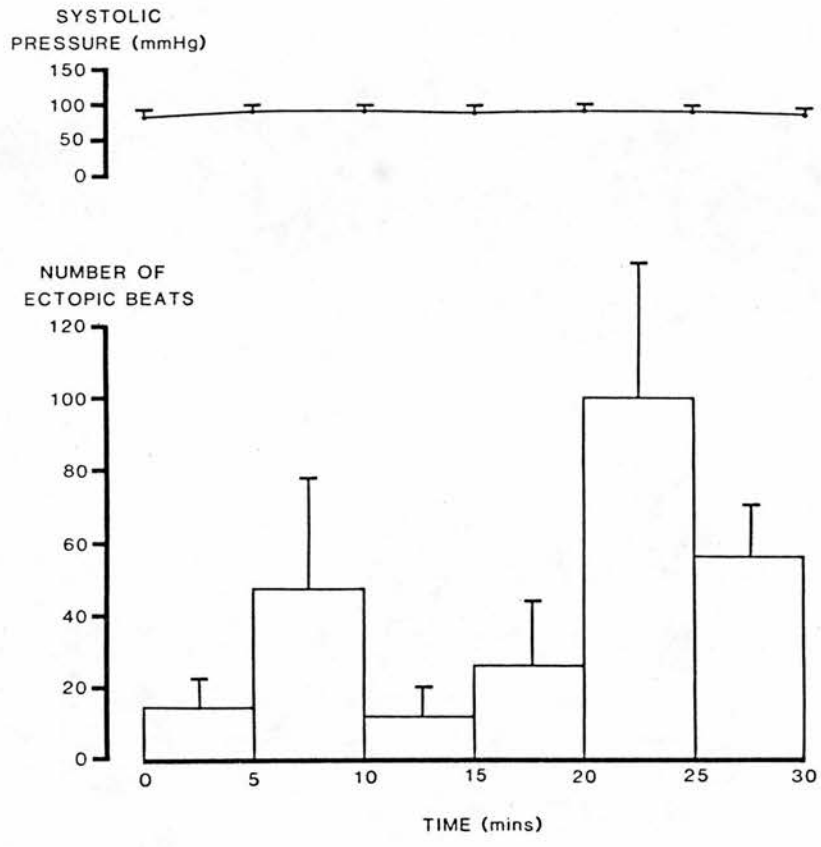


Figure 1.

Mean number of ventricular ectopic beats occurring in each 5 min period following the production of a critical stenosis of the left anterior descending coronary artery in 12 anaesthetized greyhounds. The histogram shows the characteristic biphasic distribution with peaks at 5-10 min (1a) and 20-25 min (1b).

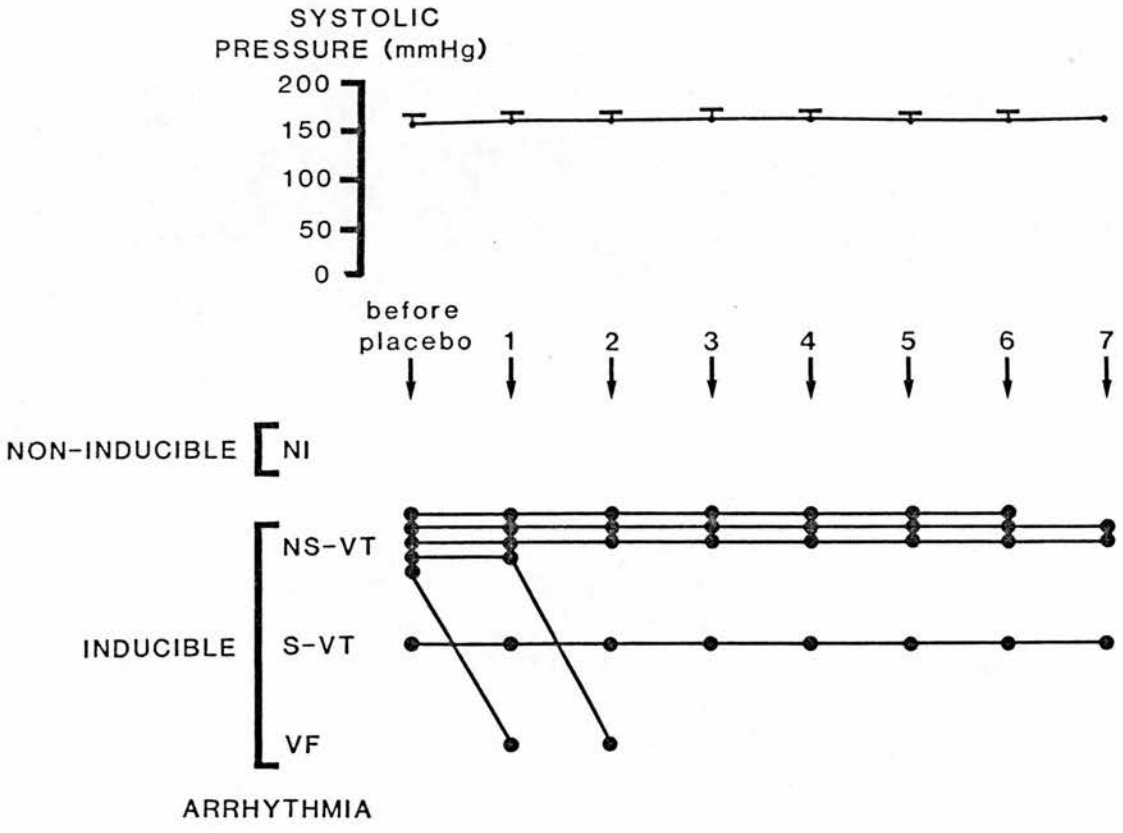


Figure 2.

Effect of placebo (doses 1,2,3 etc) upon the arrhythmias produced by programmed electrical stimulation in 6 conscious dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

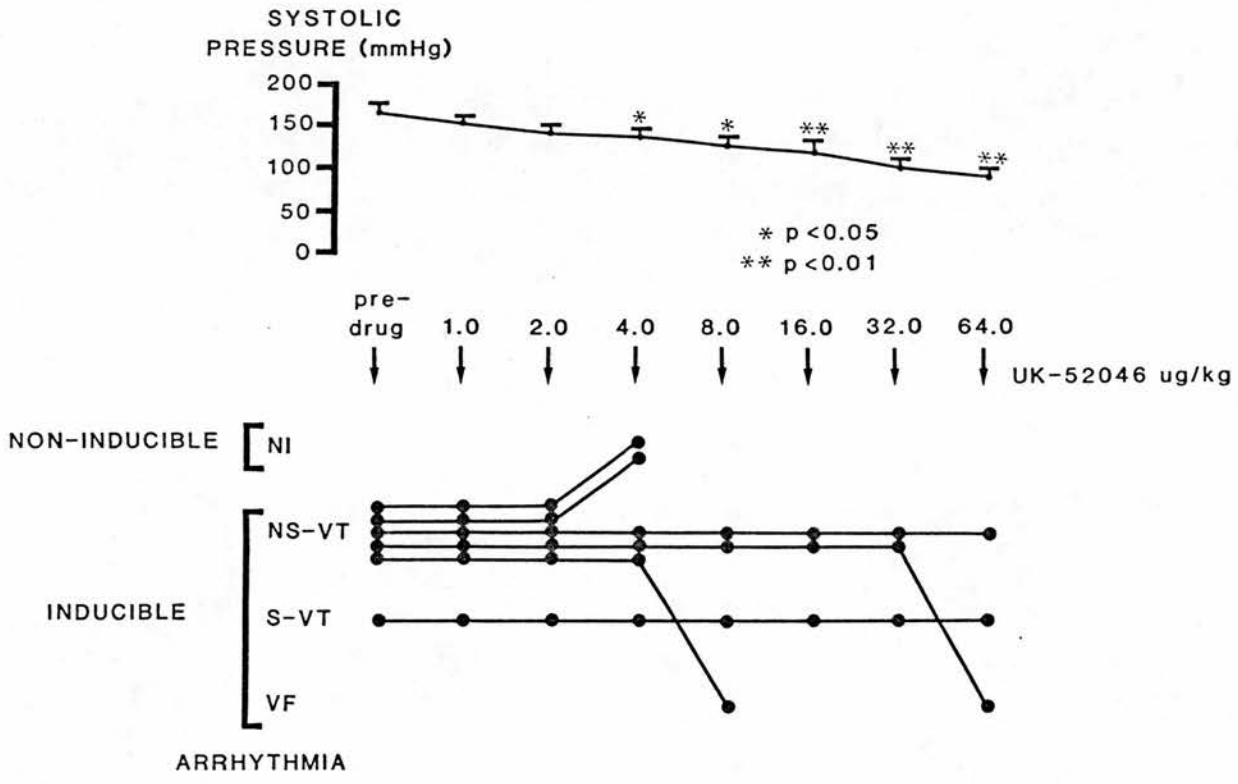


Figure 3.

Effect of increasing doses of UK-52046 upon the arrhythmias produced by programmed electrical stimulation in 6 conscious dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

Chapter 7. A COMPARISON OF THE EFFECTS OF ATENOLOL AND MEXILETINE ON EXPERIMENTAL ARRHYTHMIAS PRODUCED BY PROGRAMMED ELECTRICAL STIMULATION

Introduction

Reference has already been made to the studies with various class 1 antiarrhythmic drugs which showed that, whilst these agents were capable of suppressing the acute and chronic ventricular arrhythmias associated with myocardial infarction, none demonstrated any associated reduction in mortality. In the same chapter (chapter 2.4), attention was drawn to the trials which provided good evidence that the use of β–adrenergic receptors in the post-infarction period was associated with a reduction in both reinfarction and sudden death. The purpose of this study was to evaluate the antiarrhythmic properties of mexiletine, a commonly used class 1 antiarrhythmic agent, and atenolol, a β-adrenergic receptor antagonist, in experimental arrhythmias produced by programmed electrical stimulation. By so doing, it was intended to see whether the clinical findings described above could be reproduced in this particular model, and if so, if they could be attributed to any electrophysiological characteristics of the drugs under study.

Methods

The surgical procedures, stimulation protocol and measurement of electrophysiological variables are similar to those employed for the study described in chapter 6. Methodological details are given in chapter 4. Since a number of studies with

programmed electrical stimulation were carried out simultaneously, one control group serves for the comparison of all drug data in antiarrhythmic studies with inducible dogs.

Drugs Used

Mexiletine hydrochloride (Boehringer Ingelheim Laboratories)

Atenolol hydrochloride (Stuart Pharmaceuticals)

Drugs were freshly prepared by dissolution in 0.9%saline. All doses expressed are in terms of the hydrochloride.

Statistics

Details covered in chapter 4.

Results

The study comprises a total of 18 dogs. Three groups of six inducible dogs were randomly allocated to receive increasing intravenous doses of atenolol, mexiletine or placebo.

For the 6 dogs in the placebo group, programmed stimulation resulted in a reproducible NS-VT in 5 dogs and a S-VT in one prior to treatment. Of the 5 dogs with NS-VT, 3 remained inducible when stimulated after each of 7 doses of placebo; the other 2 developed VF when stimulated 5 min after the first dose of placebo. The dog with S-VT remained in this arrhythmia throughout the duration of the experiment (figure 6.2).

Of the 6 dogs administered atenolol (figure 7.1), 5 had a reproducible NS-VT while one was in a S-VT prior to treatment. Of the 5 with NS-VT, 3 became non-inducible after doses of 8 ug/kg, 64 ug/kg and 128 ug/kg respectively, one was still inducible after 256 ug/kg and one died when stimulated after 1 ug/kg. The dog with S-VT became non-inducible after 256 ug/kg. No adverse effects were apparent at the doses used.

In the mexiletine group all 6 dogs had a reproducible NS-VT at the start of the experiment. Of these, 5 remained inducible after doses of up to 16 mg/kg and one dog died when stimulated after 4 mg/kg (figure 7.2). All the dogs which received 16 mg/kg exhibited generalised rigidity which prevented continuation of the experiment.

Statistical analysis revealed that atenolol was significantly ($p<0.05$) better than placebo in abolishing arrhythmias produced by programmed electrical stimulation and protecting against VF. The difference between mexiletine and placebo was not statistically significant.

Blood Pressure and Heart Rate

In the placebo group mean initial systolic pressure for the 6 dogs was 155 $\pm$ 6.9 (mean $\pm$ SEM) mmHg. The corresponding heart rate was 114.2 $\pm$ 12.0 per min. Neither value altered significantly during the experiment (figure 6.2).

Mean intial systolic pressure for the group receiving atenolol was 162 $\pm$ 9.0 mmHg; after 256 ug/kg of drug this had

fallen to 147.5 ± 27.4 mmHg but the drop was not significant (figure 7.1). Heart rate, however, fell from an initial 110.4 ± 7.3 per min to 90.4 ± 6.5 per min ($p<0.05$ compared with placebo).

Mean initial systolic pressure for the mexiletine group was 165.5 ± 10.7 mmHg and did not vary significantly during the course of the experiment. Mean initial heart rate in this group was 119.0 ± 12.5 per min; this did not vary significantly with doses of up to 8 mg/kg of mexiletine but after 16 mg/kg increased to 176.5 ± 11.5 per min ($p<0.05$ compared with placebo).

Electrophysiological Measurements

The effects of placebo upon the PR interval, QRS duration, QT_c and refractory periods are summarised in table 6.2. The pre-treatment figures for placebo were not significantly different from the corresponding values for either drug group (table 7.1). There was no significant change in any parameter as the result of treatment with mexiletine (table 7.1), but atenolol resulted in a significant ($p<0.05$) prolongation of the ERP (0.115 ± 0.005 sec before treatment to 0.13 ± 0.005 sec after drug). FRP was also noted to lengthen (0.175-0.185 sec) but in this case the change was not significant (see table).

Atenolol

Parameter	Before Drug	After Drug
QT_C	0.31 ± 0.015	0.29 ± 0.005
QRS	0.08 ± 0.005	0.075 ± 0.005
PR	0.115 ± 0.005	0.12 ± 0.01
ERP	0.115 ± 0.005	0.13* ± 0.005
FRP	0.175 ± 0.005	0.185 ± 0.01

Mexiletine

Parameter	Before Drug	After Drug
QT_C	0.315 ± 0.005	0.305 ± 0.01
QRS	0.075 ± 0.005	0.075 ± 0.005
PR	0.11 ± 0.005	0.105 ± 0.005
ERP	0.115 ± 0.005	0.115 ± 0.005
FRP	0.16 ± 0.005	0.165 ± 0.005

Table 7.1

Effects of atenolol and mexiletine on the corrected QT interval (QT_C), QRS duration (QRS), PR interval (PR) and effective (ERP) and functional (FRP) refractory periods in 2 groups of 6 dogs. Results are expressed in seconds (mean ± SEM). *$p<0.05$

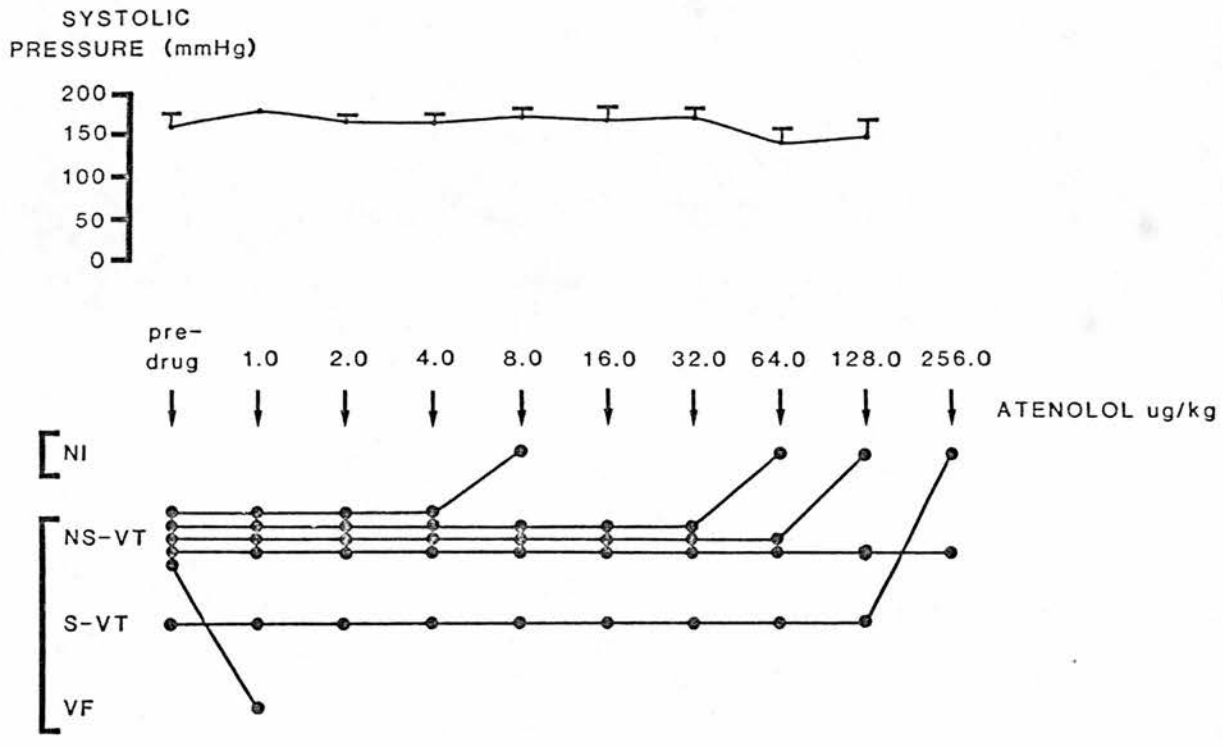


Figure 1.

Effect of atenolol upon the arrhythmias produced by programmed electrical stimulation in 6 conscious dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

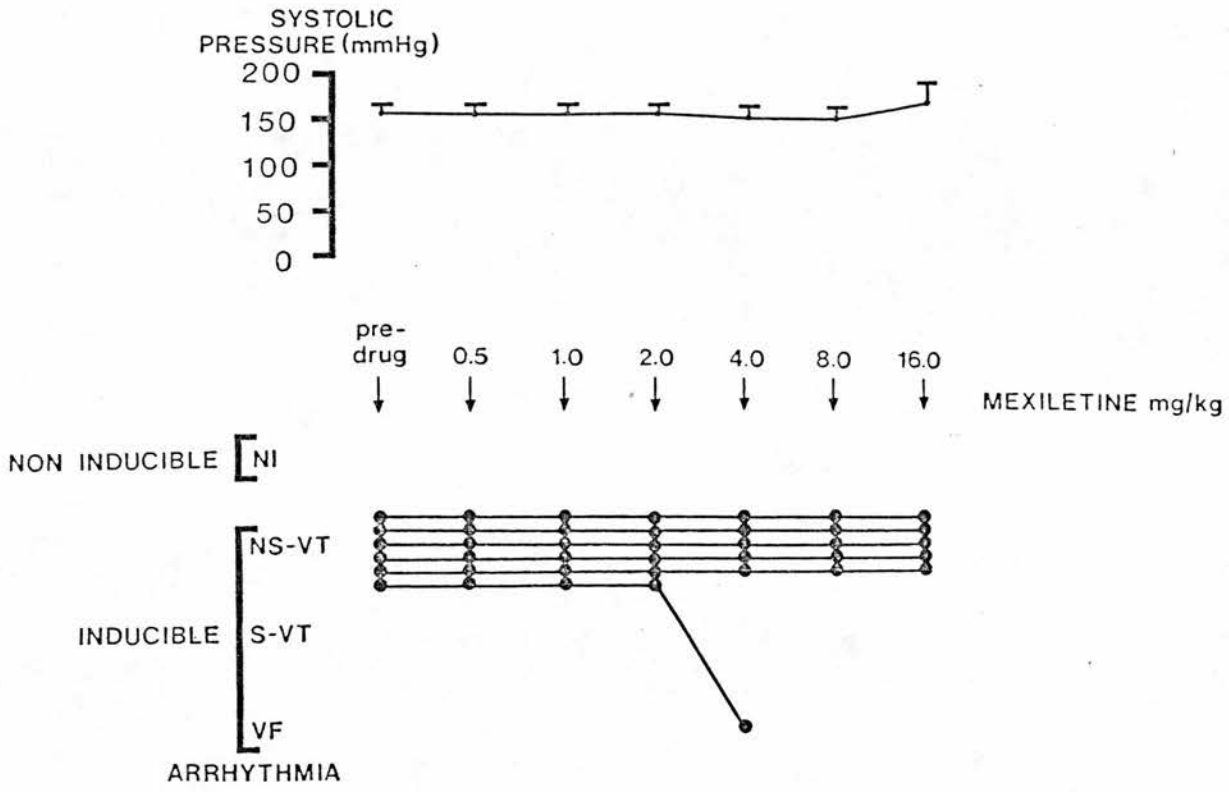


Figure 2.

Effect of mexiletine upon the arrhythmias produced by programmed electrical stimulation in 6 conscious dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

Chapter 8. EFFECTS OF THE ENANTIOMERS OF TOCAINIDE AND MEXILETINE ON EXPERIMENTAL ARRHYTHMIAS PRODUCED BY PROGRAMMED ELECTRICAL STIMULATION

Introduction

Tocainide (2-amino-2',6'-propionoxylidide HCl) is a class 1b antiarrhythmic agent, structurally related to lignocaine but with high bioavailability and a long duration of action after oral administration. The main indication for its use in man is in the treatment of intractable ventricular arrhythmias (Zipes and Troup, 1978). The drug is used clinically as a racemic mixture, but it has been shown that the R (-) enantiomer is up to three times as potent as the S (+) enantiomer in protecting against chloroform-induced fibrillation in mice (Byrnes et al, 1979). Similar, but less marked differences in enantiomeric potency were also seen in the arrhythmia occurring 24 hours after coronary artery ligation in dogs. Additional studies have suggested differences in the metabolism and/or excretion of the 2 enantiomers, resulting in lower plasma concentrations of the R enantiomer both in animal (Gal et al, 1982) and clinical (Sedman et al, 1984) studies

The purpose of this study was to investigate the antiarrhythmic and electrophysiological effects of the racemic mixture of tocainide and its enantiomers on experimental arrhythmias produced by programmed electrical stimulation. During the course of these experiments, we were fortunate enough also to acquire a limited supply of the enantiomers of mexiletine, and so it was decided to extend the study to include an analysis of the antiarrhythmic potential of the S (+) and R (-) enantiomers of

mexiletine.

Methods

The surgical procedures, stimulation protocol and measurement of electrophysiological variables are similar to those employed for the studies described in chapters 6 and 7. Measurement of blood pressure in the conscious dog was not included in the protocol of these studies, otherwise methods are as described in chapter 4.

Drugs Used

Racemic (SR) tocainide

R (-) tocainide S (+) tocainide

R (-) mexiletine S (+) mexiletine

Tocainide and its enantiomers were provided by Hassle laboratories (a subsidiary of AB Astra), Sweden. The enantiomers were prepared (99.6% purity) by fractional crystallization of the parent compound using di-p-toluyl-d and di-p-toluyl-l tartaric acid (Byrnes et al, 1979). Solutions of each drug were freshly prepared by dissolution of the hydrochloride powder in 0.9% saline. The enantiomers of mexiletine were provided by Boehringer-Ingelheim (Canada) Limited.

Statistics

Details covered in chapter 4.

Results

The first part of the study comprised three groups of six inducible dogs which were randomly allocated to receive increasing (0.5, 1.0, 2.0 etc. mg/kg) intravenous doses of SR tocainide, S tocainide, or R tocainide .

In the group which received racemic tocainide, 4/6 had an initial NS-VT and 2/6 were in S-VT (figure 8.1). Of the 4 with NS-VT, one became non-inducible after 16.0 mg/kg of drug, one developed S-VT when stimulated after 16.0 mg/kg of drug but reverted to a NS-VT after 32.0 mg/kg and 2 died when stimulated after 0.5 mg/kg and 4.0 mg/kg respectively. The 2 dogs with S-VT became non-inducible after 16.0 mg/kg and 32.0 mg/kg respectively (figure 8.1). The mean effective dose of SR tocainide for the 3 dogs which became non-inducible in this group was 21.3 mg/kg (range 16-32 mg/kg).

In the group receiving S tocainide, 3/6 dogs had a NS-VT and 3/6 were in S-VT at the start of the experiment (figure 8.2). Of the 3 with NS-VT, 2 became non-inducible after 0.5 mg/kg and 4.0 mg/kg respectively and one still demonstrated a NS-VT when stimulated after 32.0 mg/kg. Of the 3 with S-VT, 2 became non-inducible after 8.0 and 16.0 mg/kg respectively and in the third

the S-VT degenerated into VF after 32.0 mg/kg. The mean effective dose of S tocainide for the 4 dogs which became non-inducible in this group was 7.1 mg/kg (range 0.5-16.0 mg/kg).

In the group receiving R tocainide, 5/6 dogs had a NS-VT and one was in S-VT at the start of the experiment (figure 8.2). Of the 5 with NS-VT, 4 became non-inducible after doses of 0.5 mg/kg, 0.5 mg/kg, 4.0 mg/kg and 32.0 mg/kg of drug. The dog with S-VT became non-inducible after 8.0 mg/kg of drug. The mean effective dose of R tocainide for the 5 dogs which became non-inducible in this group was 9.0 mg/kg (range 0.5-32.0 mg/kg).

All the dogs which received 32.0 mg/kg of the racemic mixture or enantiomers became tremulous and agitated with profuse salivation. Two dogs developed generalised rigidity at this dose but recovered completely within 30 minutes.

Statistical analysis indicated that the S and R enantiomers of tocainide were significantly ($p<0.05$ and $p<0.01$ respectively) better than placebo (chapters 6,7) in abolishing ventricular arrhythmias produced by programmed stimulation and protecting against death. The difference between the racemic mixture and placebo did not reach statistical significance.

S and R mexiletine were studied in groups of 5 and 6 dogs respectively (there being insufficient isomer to complete a sixth study for the S group). All dogs had reproducible non-sustained ventricular tachycardias at the start of the experiments. In the

group receiving S mexiletine, one dog became non-inducible after 1.0 mg/kg of drug, 2 continued to demonstrate a NS-VT after doses of 8.0 mg/kg and 16.0 mg/kg respectively and one died when stimulated after 8.0 mg/kg. The fifth dog developed a sustained VT when stimulated after 16.0 mg/kg; the arrhythmia persisted for 5 min but degenerated into VF during an attempt to pace back into sinus rhythm (figure 8.4). In the group administered R mexiletine 3 dogs became non-inducible (2 after 0.5 mg/kg, one after 8.0 mg/kg), one continued to demonstrate a NS-VT after 16.0 mg/kg and 2 died when stimulated after 1.0 mg/kg and 8.0 mg/kg (figure 8.4). The same features of agitation which were apparent in the tocainide experiments also prevented the continuation of these experiments beyond 16.0 mg/kg of either enantiomer.

When subjected to statistical analysis, these results failed to demonstrate any significant antiarrhythmic protection when either enantiomer was compared with placebo. The same analysis, when applied to racemic mexiletine, had also failed to demonstrate antiarrhythmic efficacy (chapter 7).

Electrophysiological Measurements

The effects of placebo upon the PR interval, QRS duration, QT_C and refractory periods are summarised in table 6.2. The pre-treatment figures for placebo were not different from the corresponding values for tocainide or its enantiomers (table 8.1) or either enantiomer of mexiletine (table 8.2). Similarly, there was no

SR tocainide

Parameter	Before Drug	After Drug
QT_C	0.29 ± 0.01	0.27 ± 0.02
QRS	0.09 ± 0.005	0.08 ± 0.005
PR	0.12 ± 0.005	0.11 ± 0.01
ERP	0.12 ± 0.005	0.13 ± 0.01
FRP	0.17 ± 0.005	0.17 ± 0.005

S tocainide

Parameter	Before Drug	After Drug
QT_C	0.28 ± 0.01	0.28 ± 0.01
QRS	0.07 ± 0.005	0.07 ± 0.005
PR	0.12 ± 0.01	0.12 ± 0.01
ERP	0.125 ± 0.005	0.13 ± 0.005
FRP	0.165 ± 0.01	0.17 ±0.01

R tocainide

Parameter	Before Drug	After Drug
QT_C	0.31 ± 0.01	0.30 ± 0.01
QRS	0.07 ± 0.005	0.07 ± 0.005
PR	0.11 ± 0.005	0.11 ± 0.005
ERP	0.125 ± 0.005	0.125 ± 0.005
FRP	0.18 ± 0.01	0.18 ± 0.01

Table 8.1

Effects of SR tocainide, S tocainide and R tocainide on the corrected QT interval (QT_C), QRS duration (QRS), PR interval (PR) and effective (ERP) and functional (FRP) refractory periods in 3 groups of 6 dogs. Results in seconds (mean ± SEM).

S mexiletine

Parameter	Before Drug	After Drug
QT_C	0.30 ± 0.015	0.285 ± 0.005
QRS	0.07 ± 0.005	0.07 ± 0.005
PR	0.11 ± 0.005	0.11 ± 0.005
ERP	0.115 ± 0.005	0.12 ± 0.005
FRP	0.165 ± 0.005	0.165 ±0.01

R mexiletine

Parameter	Before Drug	After Drug
QT_C	0.305 ± 0.01	0.31 ± 0.015
QRS	0.08 ± 0.005	0.075 ± 0.005
PR	0.11 ± 0.005	0.105 ± 0.005
ERP	0.12 ± 0.005	0.125 ± 0.005
FRP	0.165 ± 0.005	0.17 ± 0.01

Table 8.2

Effects of S and R mexiletine on the corrected QT interval (QT_C), QRS duration (QRS), PR interval (PR) and effective (ERP) and functional (FRP) refractory periods in 3 groups of 6 dogs.
Results in seconds (mean ± SEM).

significant change as the result of treatment with any of the drugs under study (tables 8.1, 8.2).

The effective refractory period was defined as the shortest coupling interval capable of producing conducted beats. The functional refractory period was the minimum time between

conducted beats (chapter 4.2.3). When the inter-response interval was plotted against the inter-stimulus interval the graph displayed a characteristic appearance (figure 8.3). The bottom of the 'hockey stick' represents the slowing in conduction which occurs when closely-coupled extrastimuli propagate at a time interval close to the relative refractory period of the intervening tissue. In these experiments FRP acted as an index of conduction within the ventricular myocardium (Simson et al, 1979), and exhibited no change after treatment with any of the preparations of tocainide or mexiletine.

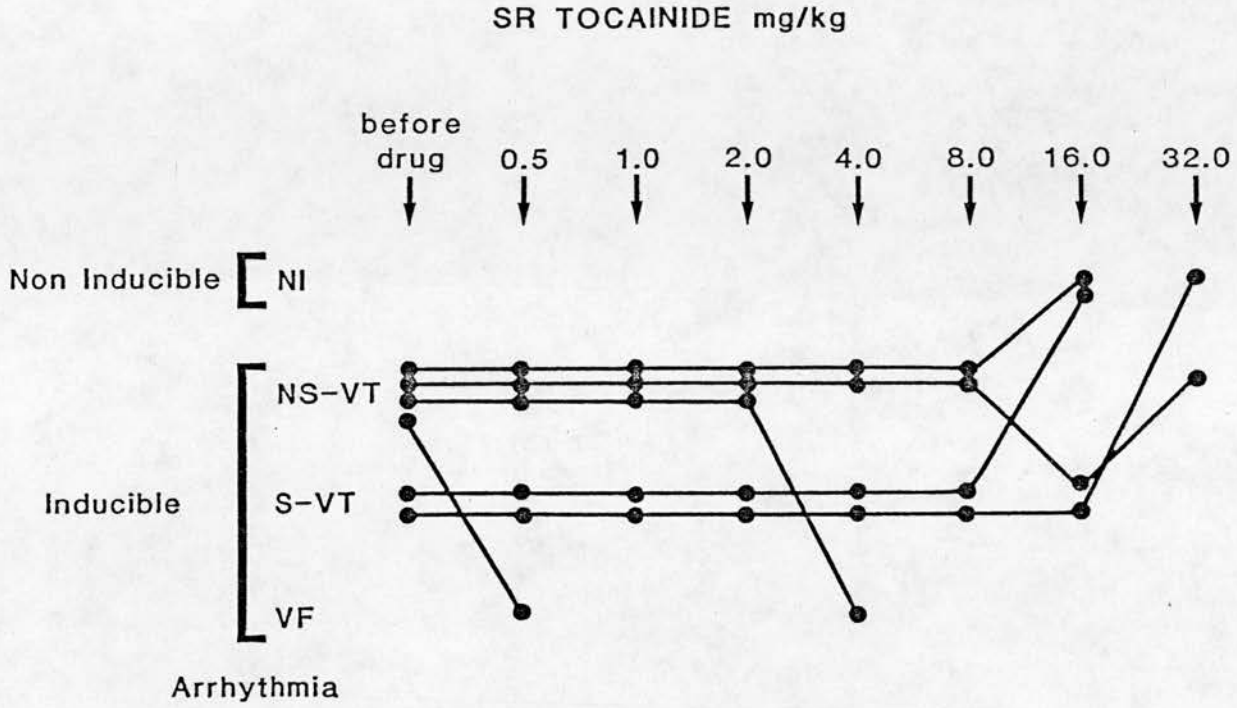


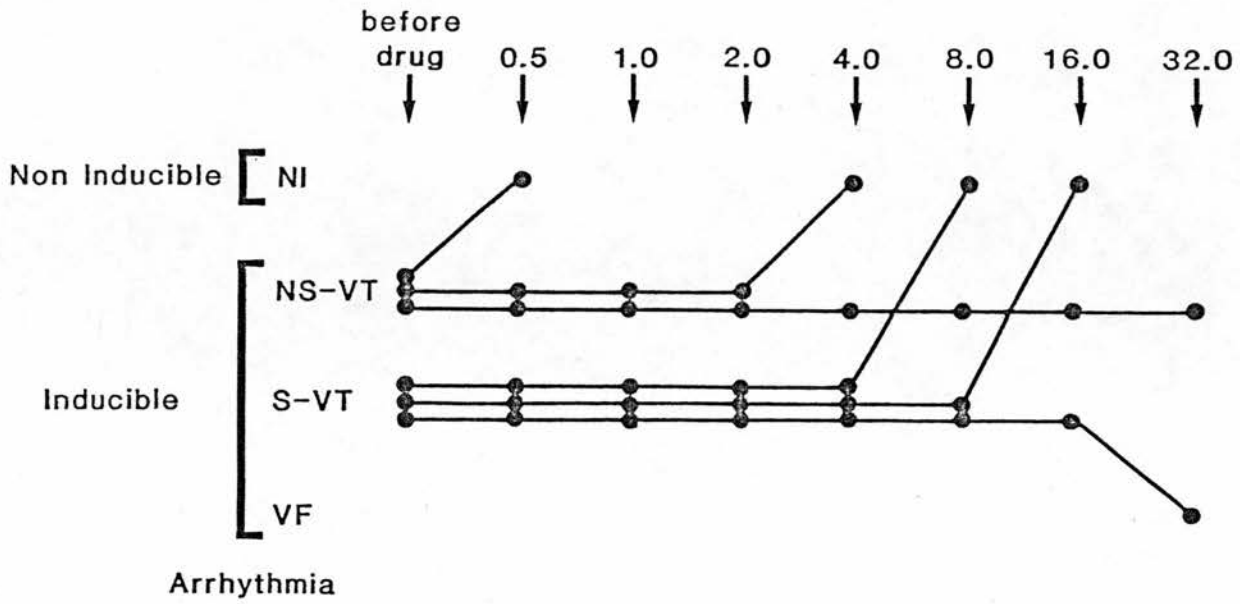
Figure 1.

Effect of SR tocainide upon the arrhythmias produced by programmed electrical stimulation in 6 conscious dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation



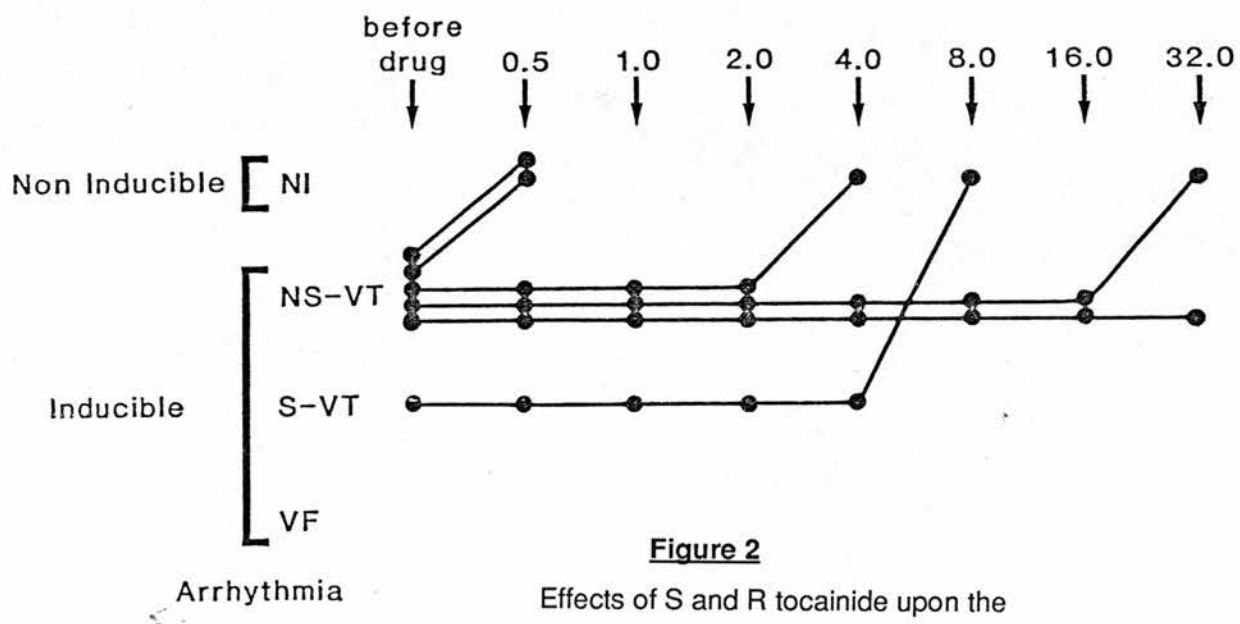


Figure 2

Effects of S and R tocainide upon the arrhythmias produced by programmed electrical stimulation in 2 groups of 6 dogs.

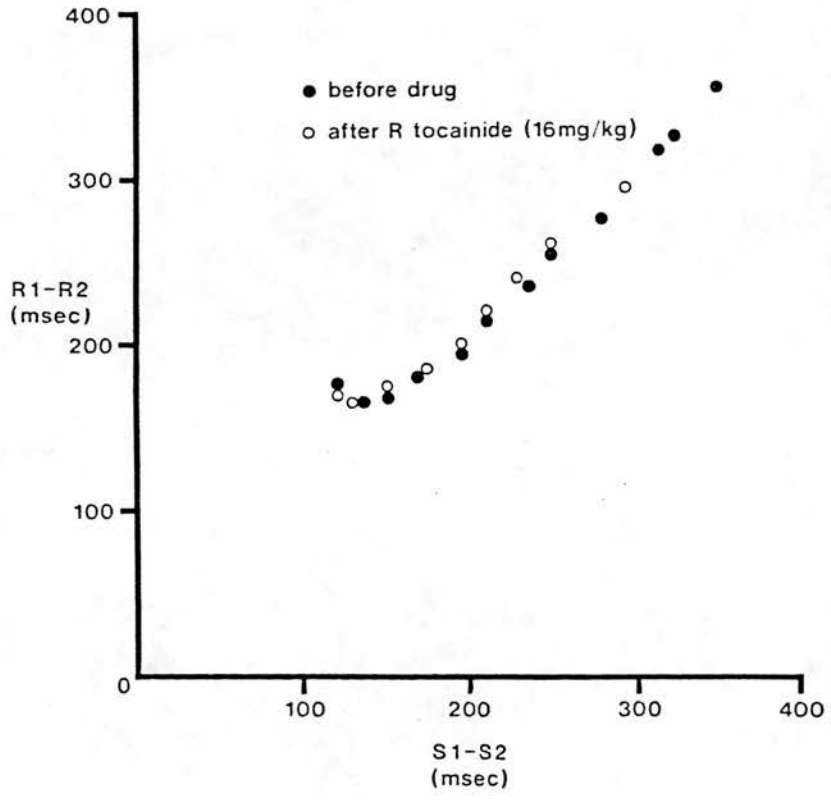
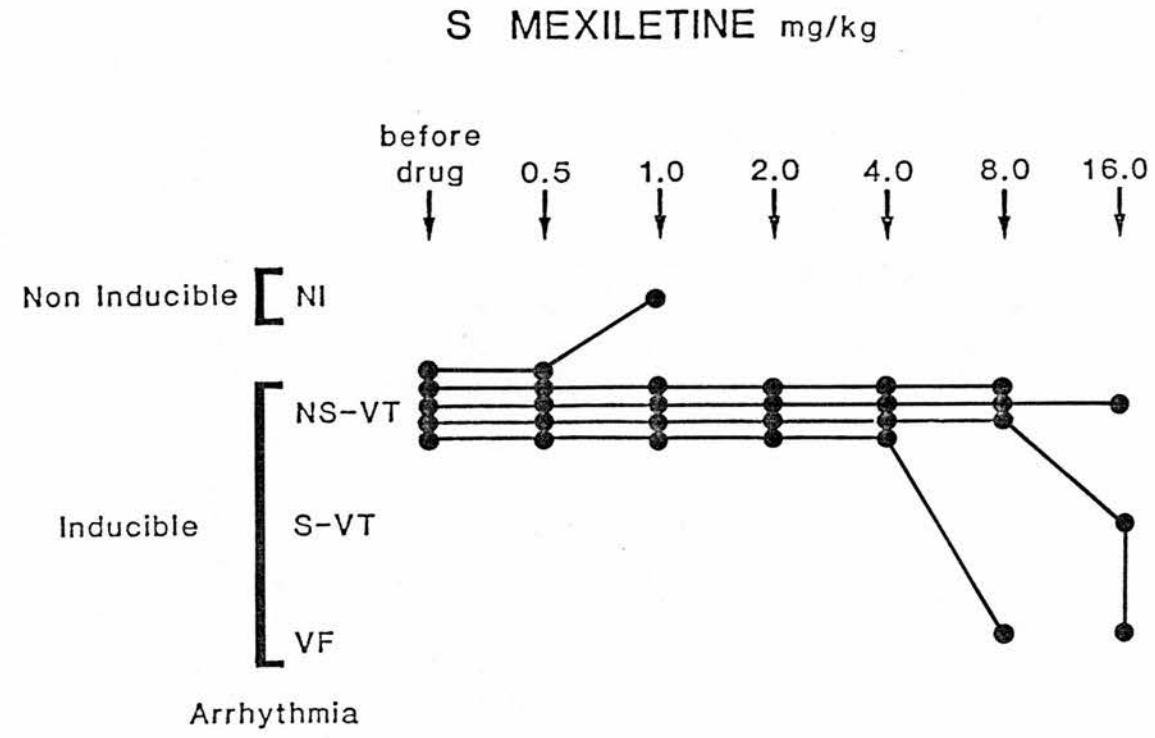


Figure 3

Relationship between effective refractory period (ERP) and functional refractory period (FRP) in a conscious dog during programmed electrical stimulation. ERP was taken as the shortest S1-S2 interval which produced a ventricular response. FRP was taken as the shortest R1-R2 interval measured during the pacing protocol.



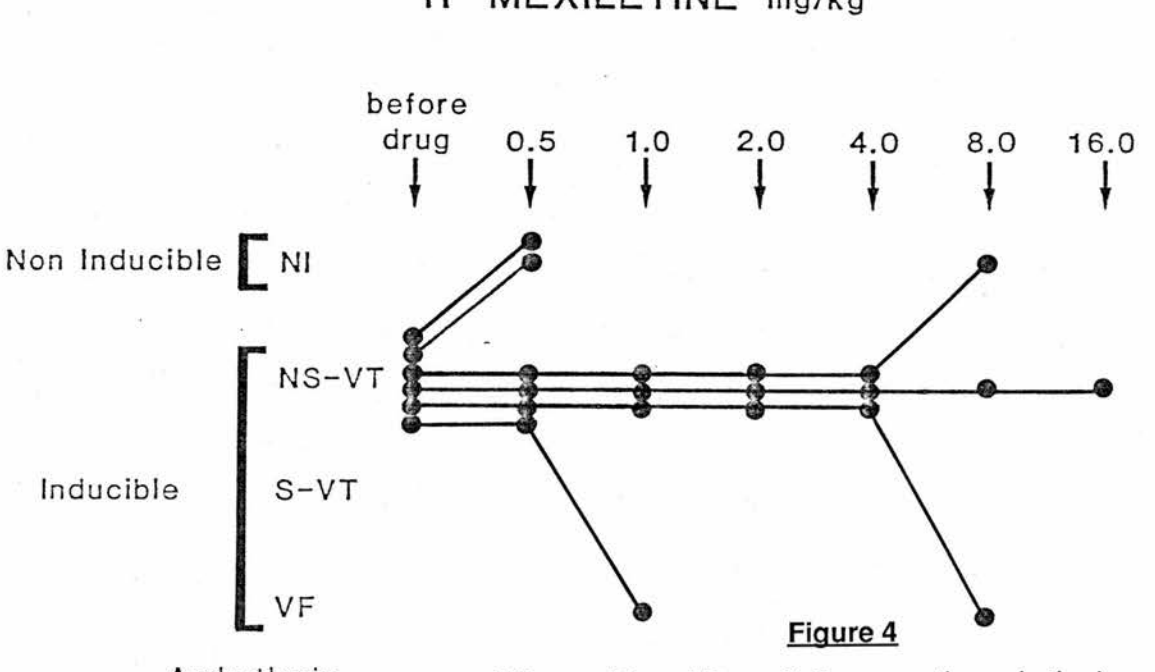


Figure 4

Effects of S and R mexiletine upon the arrhythmias produced by programmed electrical stimulation.

Chapter 9. THE PROARRHYTHMIC EFFECTS OF CIMETIDINE, RANITIDINE AND QUINIDINE

Introduction

Histamine is a vasoactive amine found in mast cells throughout the body. Although a physiological effect on the heart has not been described, histamine is present in large quantities in cardiac tissue (Vugman and Rocha e Silva, 1966) and has been shown to increase sinus rate and contractility, prolong AV conduction and enhance automaticity in an isolated preparation (Levi, 1972). Further studies have characterized these effects into those mediated respectively by H_1 (prolonged conduction) and H_2 (positive inotropic and chronotropic effects and enhanced automaticity) receptors (Levi et al, 1976). After the discovery that histamine levels were significantly increased in patients after myocardial infarction (Rai et al, 1976), it was suggested that histamine could induce arrhythmias (Levi et al, 1981), especially under ischaemic conditions (Cameron et al, 1983 (a)). Following the development of the H_2-specific antagonists in the late 1960's, Black and colleagues showed that the characteristic cardiac effects of histamine in isolated preparations could be selectively blocked by these drugs (Black et al, 1972). The subsequent reports of sinus bradycardia associated with cimetidine (Reding et al, 1977; Bournerias et al, 1978; Stimmesse et al, 1978; Karch and Becker, 1980; Matthews et al, 1982) and ranitidine (Camarri et al, 1982; Shah, 1982) could therefore be explained from a knowledge of the drugs' pharmacological actions in blocking cardiac H_2 receptors. Whilst some have considered these effects antiarrhythmic (Ligumsky et al,

1978; Cameron et al, 1983 (b)), there have been several reports linking the intravenous use of H_2 receptor antagonists with serious cardiac arrhythmias (Cohen et al, 1979; MacMahon et al, 1981; King, 1981; Lineberger et al, 1985) and even sudden death (Shaw et al, 1980). The purpose of the present study was to investigate the proarrhythmic and electrophysiological effects of cimetidine and ranitidine in the chronic canine model of myocardial infarction which has already been described for the investigation of antiarrhythmic effects (chapters 6-8). Furthermore, in order to validate the model's suitability for proarrhythmic studies, we concurrently investigated the effects of quinidine, a class 1 antiarrhythmic agent with known proarrhythmic properties (Selzer and Wray, 1964; Reynolds and Vander Ark, 1976; Jenzer and Hagemeijer, 1976; Goldstein et al, 1984).

Methods

The surgical procedures, stimulation protocol and measurement of electrophysiological variables are similar to those employed for the study described in chapters 6-8, with the exception that this study involved the use of dogs which were non-inducible when challenged with programmed stimulation. Methodological details are given in chapter 4.

Drugs Used

Quinidine sulphate (Sigma Chemical Company)

Cimetidine base (Smith, Klein and French Laboratories)

Ranitidine hydrochloride (Glaxo Laboratories)

Each drug was available in powder form and was freshly prepared by dissolution in 0.9% saline at room temperature.

Statistics

Details covered in chapter 4.

Results

The study comprised four groups of 6 dogs in which reproducible ventricular tachycardias had previously been induced but which were non-inducible on this occasion. The dogs were randomly allocated to receive increasing (0.5, 1.0, 2.0 etc mg/kg) intravenous doses of quinidine, cimetidine, ranitidine or placebo.

The effects of repeated stimulation in the 6 dogs in the placebo group are illustrated in figure 9.1. Four dogs remained non-inducible throughout the duration of the experiment, and dog developed a reproducible NS-VT after the fourth dose of placebo and was still inducible at the end of the experiment, and one dog developed VF when stimulated 5 min after the first dose of placebo.

In the quinidine group, 3/6 dogs developed a NS-VT, one when stimulated after 0.5 mg/kg and a further 2 when challenged after 4.0 mg/kg. Three dogs developed VF when stimulated after 4.0mg/kg, 8.0 mg/kg and 16.0 mg/kg respectively. (figure 9.2).

In the cimetidine group (figure 9.3), 4/6 dogs remained non-inducible when stimulated after doses up to 16.0 mg/kg (2 dogs) and 32.0 mg/kg (2 dogs). One dog developed a reproducible NS-VT when stimulated after 4.0mg/kg and was still inducible after 16.0 mg/kg. One dog developed VF when stimulated 5 min after 0.5 mg/kg of cimetidine. Excess salivation and restlessness were noted in all dogs which received 16.0 mg/kg, and necessitated abandoning the experiment after this dose in 3 dogs (figure 9.3).

In the ranitidine group, three of the 6 dogs studied remained non-inducible when stimulated after doses up to 4.0 mg/kg, 8.0 mg/kg and 16.0 mg/kg respectively (figure 9.4). Two dogs developed a reproducible NS-VT after 0.5 mg/kg of ranitidine; of these, one developed VF after 1.0 mg/kg and the other developed VF after 4.0 mg/kg. A third dog developed VF after 16.0 mg/kg of drug. Features of salivation and restlessness were also apparent in this group, requiring the experiment to be terminated after 4.0 mg/kg (one dog), 8.0 mg/kg (one dog) and 16.0 mg/kg (last remaining dog).

Statistical analysis indicated that there was a significant ($p<0.05$) increase in arrhythmia induction and death when quinidine was compared with placebo; no difference was observed when cimetidine or ranitidine were similarly compared.

Blood Pressure and Heart Rate

In the placebo group, mean systolic pressure for the 6 dogs at the start of the experiments was 170.0 ± 10.7 mmHg (mean ± SEM).

The corresponding value for heart rate was 117.7 ± 6.4 per min. There was no significant change in either value following 7 doses of placebo (figure 9.1).

In the quinidine group, mean initial systolic pressure for the six dogs was 150.0 ± 6.0 mmHg. After 1.0 mg/kg of drug this fell to 133.3 ± 6.0 mmHg ($p<0.05$ compared with placebo).Further falls were apparent with increasing doses of drug (figure 9.2). Heart rate rose from an initial 117.5 ± 12.2 per min to 155.2 ± 12.7 after 8.0 mg/kg ($p<0.05$ compared with placebo).

Mean initial systolic pressure for the 6 dogs in the cimetidine group was 153.3 ± 14.8 mmHg. After 32.0 mg/kg of drug this had fallen to 135.0 ± 13.6 mmHg (figure 9.3), but the difference was not statistically significant when compared with placebo. Heart rate rose from an initial 111.8 ± 9.6 per min to 164.4 ± 22.7 per min after 16.0 mg/kg ($p<0.05$) and 220.0 ± 14.7 after 32.0 mg/kg ($p<0.01$ compared with placebo).

Mean initial systolic pressure for the 6 dogs in the ranitidine group was 166.7 ± 19.2 mmHg. After 16.0 mg/kg of drug this had fallen to 145.0 mmHg, but the difference was not significant (figure 4). Mean heart rate rose from an initial 113.5 ± 7.5 per min to 150.3 ± 9.6 per min after 8.0 mg/kg ($p<0.05$) and 180.0 ± 31.9 per min after 16.0 mg/kg ($p<0.05$ compared with placebo).

Electrophysiological Measurements

The effects of placebo upon the PR interval, QRS duration, QT_C

and refractory periods are summarised in table 9.1. Mean PR for the 6 dogs before and after placebo was 0.11 sec and 0.105 sec respectively (difference not statistically significant). Similarly,there was no change in mean QRS (0.075 - 0.08 sec), mean QT_C (0.33 - 0.32 sec) or mean ERP (0.12 - 0.13 sec). FRP remained constant at 0.175 sec. The pre-treatment values in the placebo group (table 9.1) were similar to the pre-treatment values in each drug group (table 9.2). There was no significant change in PR, QRS, or QT_C as the result of treatment with cimetidine or ranitidine but QT_C increased from 0.325 ± 0.01 sec to 0.355 ± 0.005 sec after quinidine ($p<0.01$ compared with placebo). After both cimetidine and ranitidine ERP and FRP appeared to shorten. This was more marked with

Placebo

Parameter	Before Drug	After Drug
QT_C	0.33 ± 0.01	0.32 ± 0.01
QRS	0.075 ± 0.005	0.08 ± 0.005
PR	0.11 ± 0.005	0.105 ± 0.005
ERP	0.12 ± 0.01	0.13 ± 0.005
FRP	0.175 ± 0.005	0.175 ± 0.005

Table 9.1

Effects of placebo on the corrected QT interval (QT_C), QRS duration (QRS), PR interval (PR) and effective (ERP) and functional (FRP) refractory periods in a group of 6 non- inducible dogs. Results are expressed in seconds (mean ± SEM).

Quinidine

Parameter	Before Drug	After Drug
QT_C	0.325 ± 0.01	0.355*±0.005
QRS	0.07 ± 0.005	0.07 ± 0.005
PR	0.115 ± 0.005	0.115 ± 0.005
ERP	0.125 ± 0.01	0.125 ± 0.005
FRP	0.165 ± 0.005	0.16 ± 0.005

Cimetidine

Parameter	Before Drug	After Drug
QT_C	0.31 ± 0.01	0.33 ± 0.005
QRS	0.08 ± 0.005	0.07 ± 0.005
PR	0.105 ± 0.005	0.10 ± 0.01
ERP	0.13 ± 0.01	0.11 ± 0.01
FRP	0.17 ± 0.005	0.145 ±0.005

Ranitidine

Parameter	Before Drug	After Drug
QT_C	0.315 ± 0.005	0.32 ± 0.01
QRS	0.08 ± 0.005	0.07 ± 0.005
PR	0.115 ± 0.005	0.115 ± 0.005
ERP	0.135 ± 0.005	0.1†
FRP	0.175 ± 0.005	0.13 - 0.135†

Table 9.2

Effects of quinidine, cimetidine and ranitidine on the corrected QT interval (QT_C), QRS duration (QRS), PR interval (PR) and effective (ERP) and functional (FRP) refractory periods in 3 groups of 6 dogs. Results in seconds.
*$p<0.05$ (compared with placebo)
†only 3 results available

ranitidine, with ERP and FRP decreasing from 0.135 - 0.1 sec and 0.175 - 0.135 sec respectively. However, in view of the small numbers used (3 surviving programmed stimulation in the ranitidine group), this was not suitable for statistical analysis.

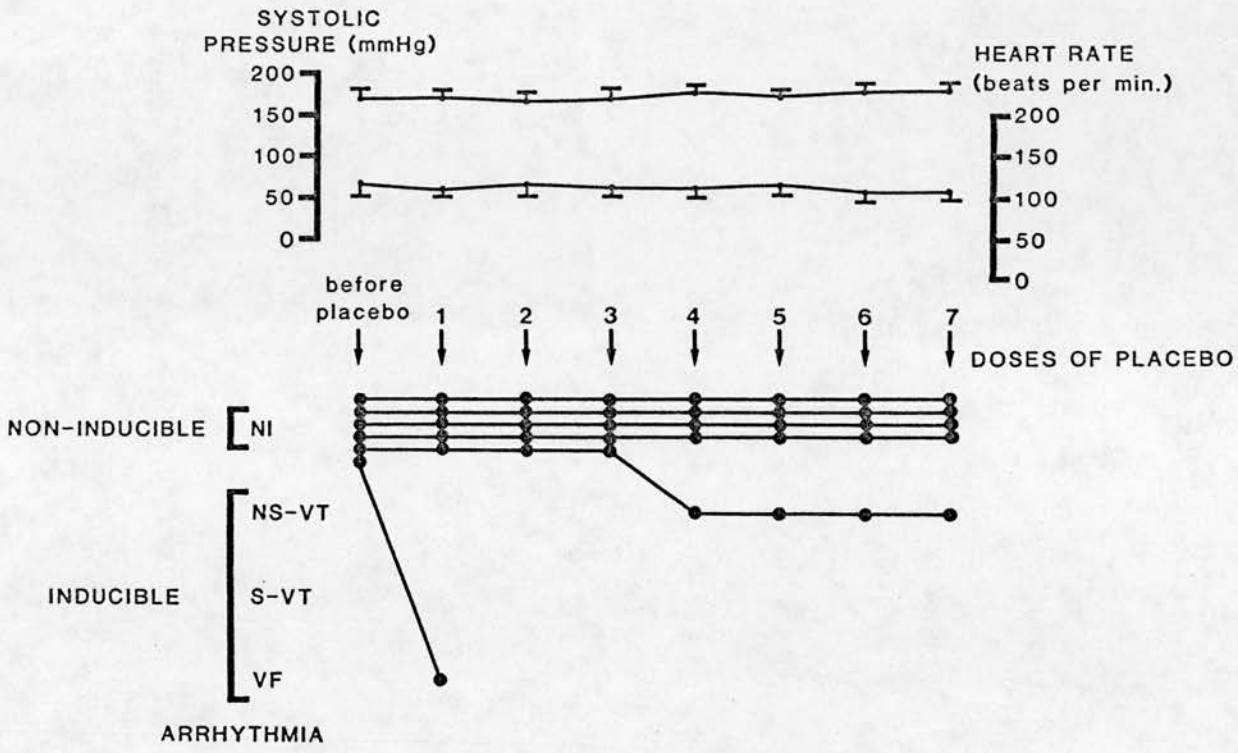


Figure 1.

Effect of placebo upon the arrhythmias produced by programmed electrical stimulation in 6 non-inducible dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

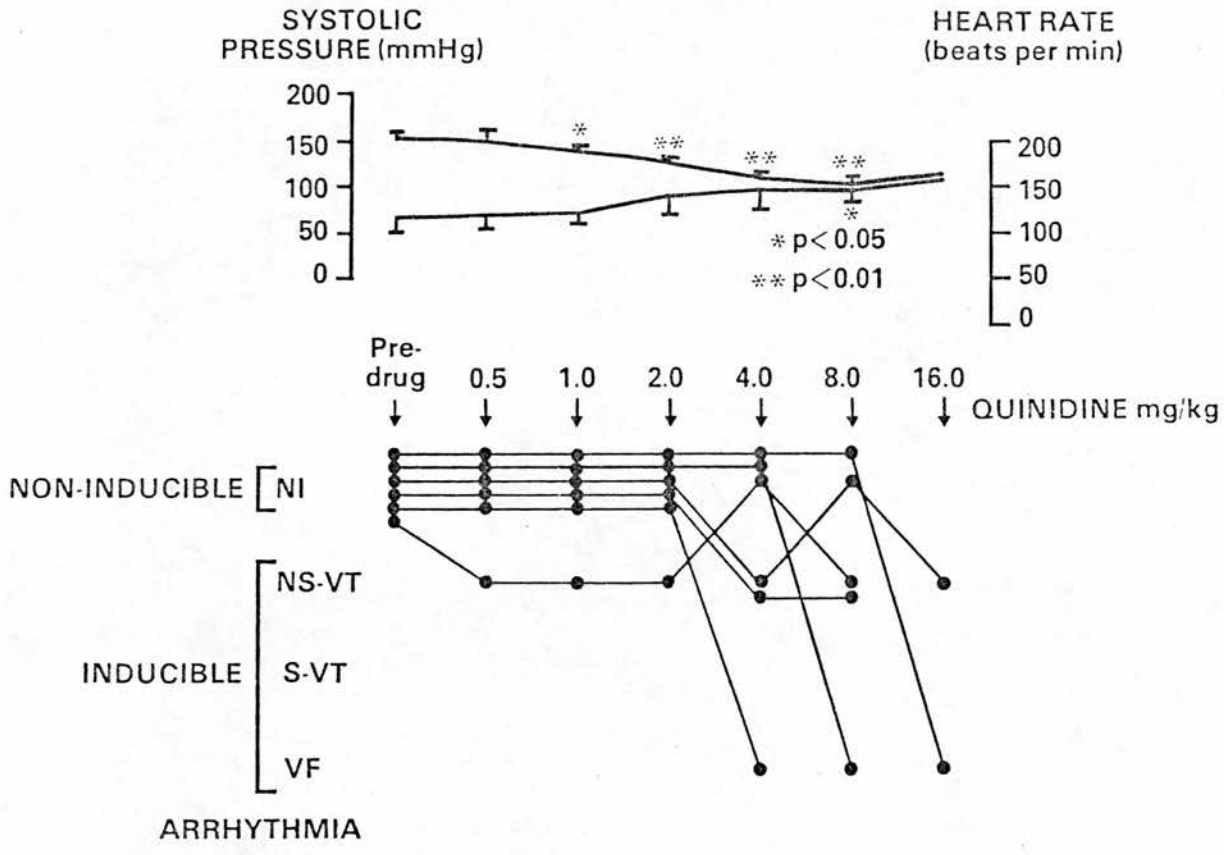


Figure 2.

Effect of quinidine upon the arrhythmias produced by programmed electrical stimulation in 6 non-inducible dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

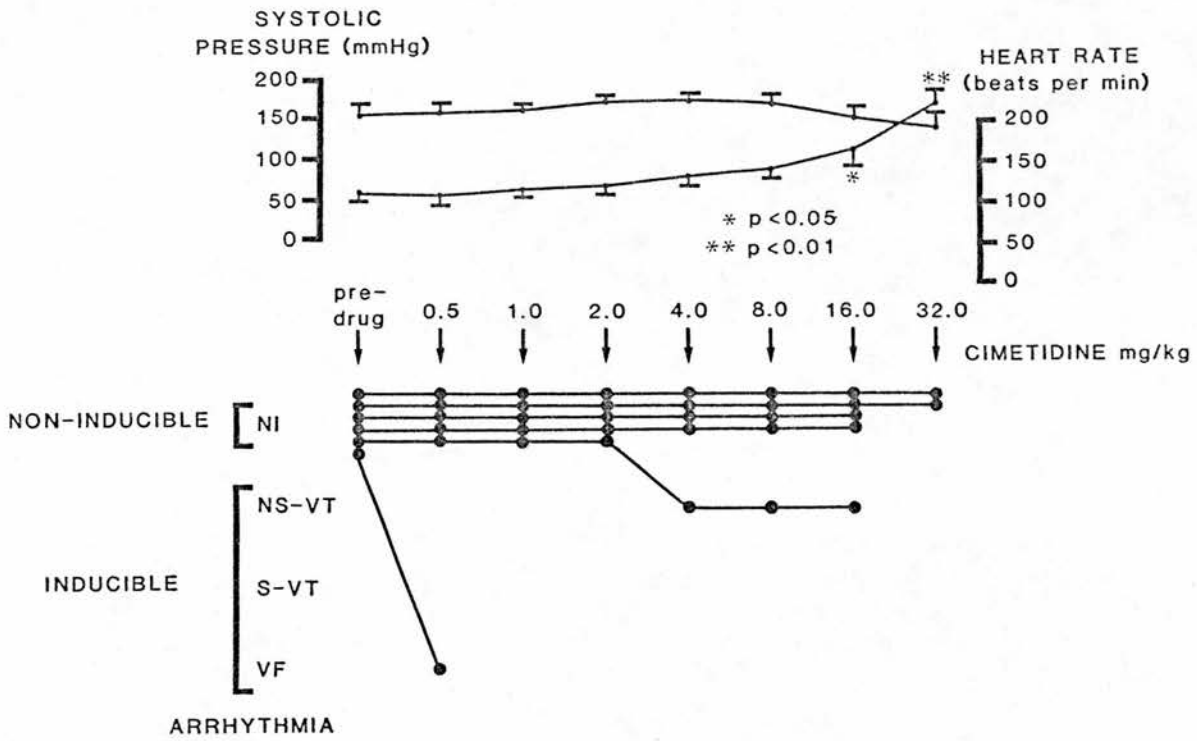


Figure 3.

Effect of cimetidine upon the arrhythmias produced by programmed electrical stimulation in 6 non-inducible dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

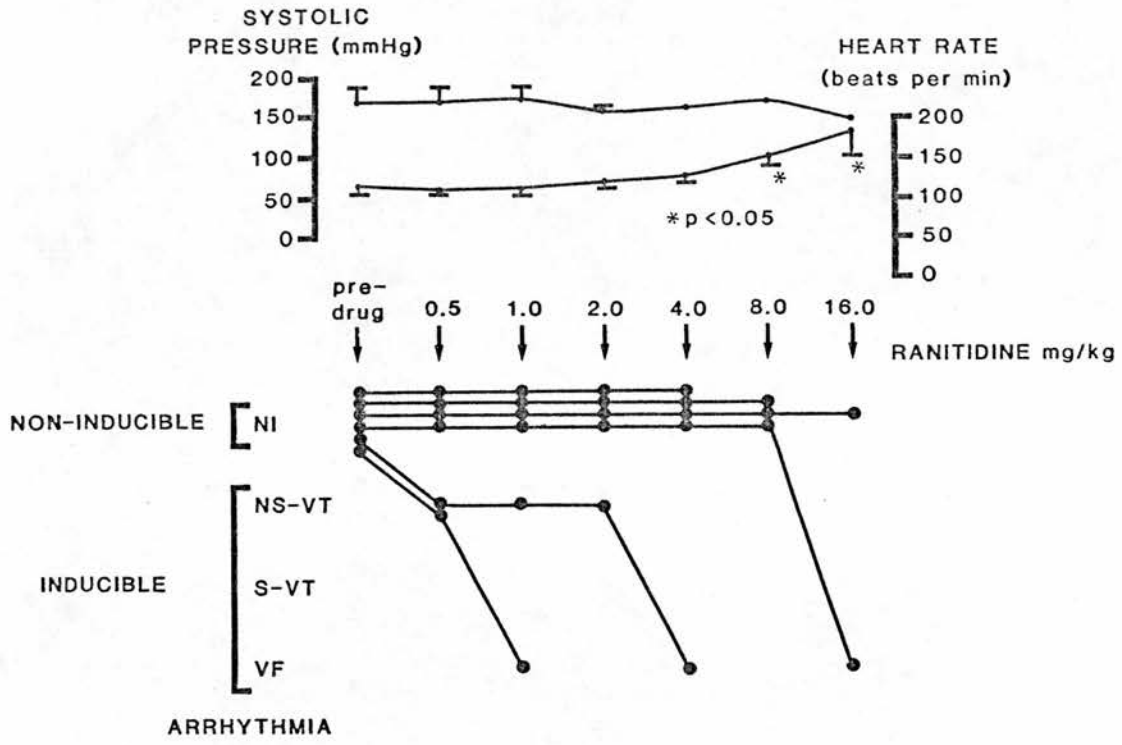


Figure 4.

Effect of ranitidine upon the arrhythmias produced by programmed electrical stimulation in 6 non-inducible dogs, 7-30 days after experimental coronary artery ligation.

NS-VT, Non-Sustained Ventricular Tachycardia

S-VT, Sustained Ventricular Tachycardia

VF, Ventricular Fibrillation

Chapter 10. INFARCT SIZES AND RELATIONSHIP TO INDUCIBILITY OF ARRHYTHMIAS DURING PROGRAMMED ELECTRICAL STIMULATION

Introduction

This chapter summarizes the results of infarct-size determinations in all the dogs which were prepared for programmed electrical stimulation during the tenure of the author's research fellowship. The purpose of so doing was primarily to establish if any relationship existed between the size of myocardial infarction and inducibility of arrhythmias on challenge with stimulation; of additional interest was to follow, with time, the macroscopic evolution and regression of the infarct itself.

Methods

The staining methods for determination of infarct size are described in chapter 4. The statistical analyses employed are covered in the same chapter.

Results

A total of 119 dogs were studied. Of these, 87 (73.1%) were alive and ambulant 24 hours after surgery. The remaining 32 (26.9%) died from various causes, of which acute ischaemic ventricular fibrillation was the most common, occurring in 20 (62.5%). Other causes included irreversible bradycardia leading to asystole (3), anaesthetic-related deaths (4), fatal haemorrhage (1) and mechanical pump failure (1). One dog pulled its pacing wires out and died from the resulting haemorrhage/tamponade and 2 animals

appeared to recover from anaesthetic but were found dead the following morning.

Of the 87 dogs which survived the immediate (24 hour) peri-operative period, 6 subsequently had to be destroyed, 3 on account of severe wound infections, 2 for vomiting and dehydration refractory to intravenous fluid replacement and one for severe subcutaneous (surgical) emphysema at 48 hours. Fifteen dogs died suddenly and unexpectedly during the first postoperative week: of these, a ruptured left ventricle was diagnosed at post-mortem in one but no cause was apparent in any of the remaining 11 which were the subject of autopsy. It may be significant that in 2 of these cases, death occurred when the dog was exercised outside on the second postoperative day in particularly cold weather. Taken as a group, these 'sudden, unexpected deaths' did not occur later than the sixth postoperative day and had a mean infarct size of 11.1 ± 1.5% of left ventricular (LV) mass.

Of the 87 dogs surviving surgery therefore, 66 (75.9%) reached programmed electrical stimulation on the seventh postoperative day (table 10.1). Of these 66, 25 died from ventricular fibrillation during the pacing protocol before the administration of drug, 36 exhibited a non-fatal arrhythmia suitable for inclusion in an antiarrhythmic drug study and 5 were deemed non-inducible by the criteria defined in chapter 4. This is illustrated in table 10.1, a flow diagram summarizing survival figures for all the dogs studied. Infarct size estimations were available in 24 of the 25 dogs which

died before drug administration and gave a mean value of 7.0 ± 1.8% of LV mass.

The fate of the 41 dogs which were entered into drug studies can be followed in table 10.1. Once a previously inducible animal became non-inducible, it was entered into a proarrhythmic drug study. If the animal survived and was found to be non-inducible when next stimulated, it was either re-operated upon or destroyed. It is interesting that, during the course of the work, 7 dogs were inducible when stimulated a week after being non-inducible.

If the dogs which died prior to receiving drug are added to those deemed inducible by the criteria established in chapter 4, and the sum expressed as a percentage of the total number of dogs stimulated, it can be seen that one week after surgery, 92.4% of the dogs were inducible. At two weeks after surgery the corresponding figure was 66.7%, with subsequent values of 64.3% and 55.6% for the third and fourth postoperative weeks respectively. When subjected to statistical analysis, these figures confirm a significant reduction in inducibility with time. The results are summarized in table 10.2, where rows 1 to 4 respectively refer to the first, second, third and fourth stimulations.

Mean infarct size for all the dogs which died (pre- or post-drug) at one week was 7.0 ± 0.5% of LV mass. The corresponding values for those dogs which died at 2 and 3 weeks were 5.8 ± 0.6% and 4.6 ± 0.8% respectively. Infarct size determinations were available for only one of the 3 dogs which died

in relation to stimulation at 4 weeks and in this case no infarct could be visualised. Statistical analysis of these results confirms a time-dependent decrease in infarct size which was of statistical significance up to one week but did not alter significantly thereafter (table 10.3).

Since dogs were not routinely destroyed for non-inducibility per se, infarct sizes for the 'non-inducible group' were calculated from those dogs which became inducible (and died) after inclusion in a proarrhythmic study or which were destroyed for repeated (successive occasions) non-inducibility. The resulting figure for infarct size in this group was therefore 5.4 ± 1.6% of LV mass. Statistical analysis failed to demonstrate a significant difference between the inducible and non-inducible groups, although both were significantly different from the 'sudden, unexpected death' group (table 10.4).

Infarcts were predominantly subendocardial in nature, as illustrated in figure 4.9. Occasionally large, transmural infarctions were apparent; although this was the case with the dog which died of a ruptured ventricle, no attempt was made to include this feature in the analysis of infarct sizes.

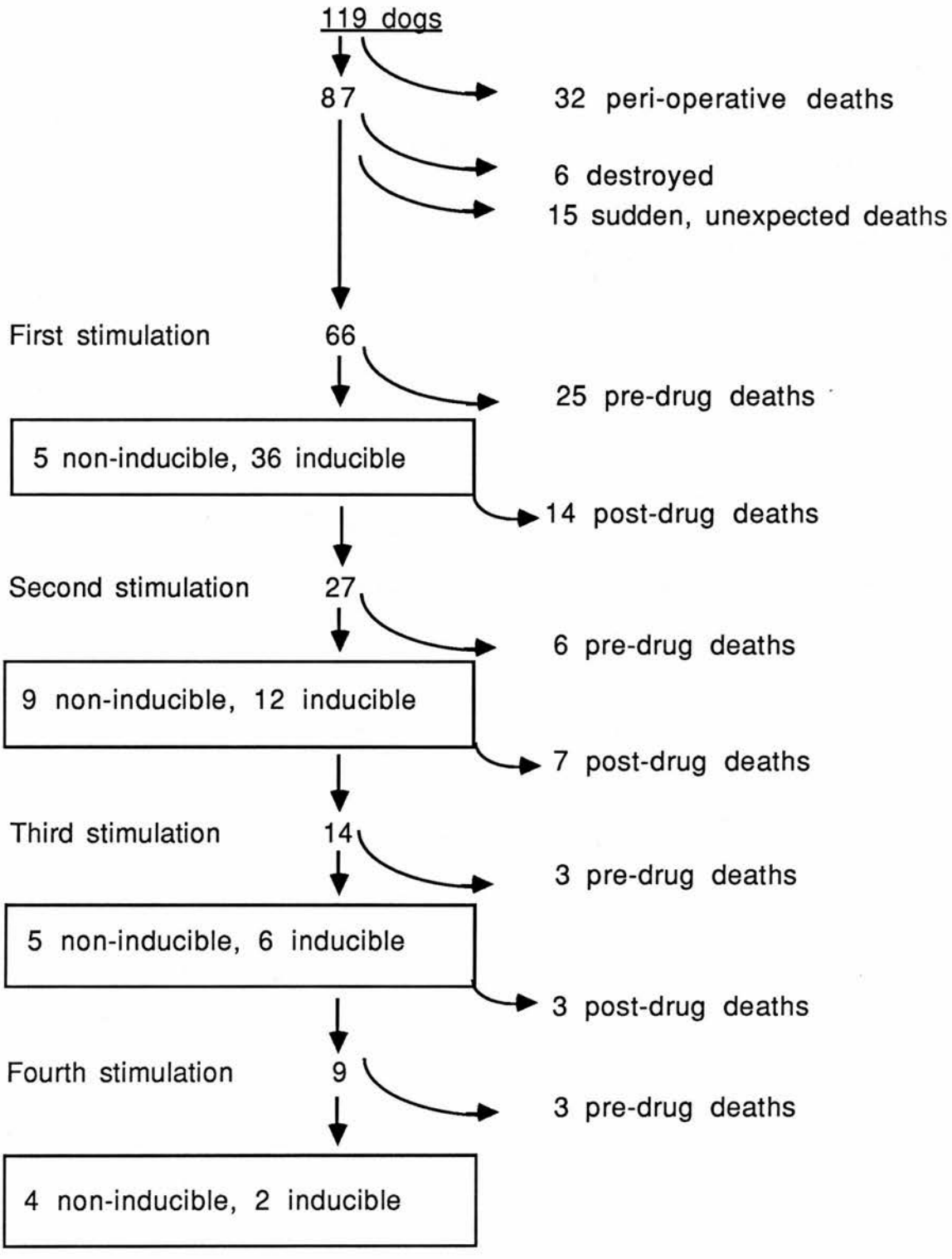


Table 10.1.
(see text for details)

Contingency Table Analysis

Summary Statistics

DF:	3	
Total Chi-Square:	14.984	p=.0018
G Statistic:	15.137	
Contingency Coefficient:	.338	
Cramer's V:	.359	

Observed Frequency Table

	Column 1	Column 2	Totals:
Row 1	61	5	66
Row 2	18	9	27
Row 3	9	5	14
Row 4	5	4	9
Totals:	93	23	116

Percents of Column Totals

	Column 1	Column 2	Totals:
Row 1	65.59%	21.74%	56.9%
Row 2	19.35%	39.13%	23.28%
Row 3	9.68%	21.74%	12.07%
Row 4	5.38%	17.39%	7.76%
Totals:	100%	100%	100%

Percents of Row Totals

	Column 1	Column 2	Totals:
Row 1	92.42%	7.58%	100%
Row 2	66.67%	33.33%	100%
Row 3	64.29%	35.71%	100%
Row 4	55.56%	44.44%	100%
Totals:	80.17%	19.83%	100%

Expected Values

	Column 1	Column 2	Totals:
Row 1	52.91	13.09	66
Row 2	21.65	5.35	27
Row 3	11.22	2.78	14
Row 4	7.22	1.78	9
Totals:	93	23	116

Table 10.2

Analysis of the relationship between inducibility and time after coronary artery ligation (key given in text)(Chi Square analysis).

One Factor ANOVA X_1: Weeks Y_1: Infarct sizes

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	247.227	82.409	7.99
Within groups	66	680.711	10.314	p = 1.0000E-4
Total	69	927.938		

Model II estimate of between component variance = 24.032

One Factor ANOVA X_1: Weeks Y_1: Infarct sizes

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Group 1	12	11.092	5.06	1.461
Group 2	39	7.015	2.878	.461
Group 3	13	5.823	2.296	.637
Group 4	6	4.617	2.056	.839

One Factor ANOVA X_1: Weeks Y_1: Infarct sizes

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Group 1 vs. 2	4.076	2.117*	4.928*	3.845
Group 1 vs. 3	5.269	2.567*	5.598*	4.098
Group 1 vs. 4	6.475	3.206*	5.42*	4.032
Group 2 vs. 3	1.192	2.054	.448	1.159
Group 2 vs. 4	2.399	2.812	.967	1.703

* Significant at 95%

One Factor ANOVA X_1: Weeks Y_1: Infarct sizes

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Group 3 vs. 4	1.206	3.165	.193	.761

Table 10.3

Analysis of the relationship between infarct size and time after coronary artery ligation (One-Way Analysis of Variance with Multiple Comparisons).

Key: Group 1, Sudden deaths within first week.

Group 2, Total deaths at first stimulation (1 week).

Group 3, Total deaths at second stimulation (2 weeks).

Group 4, Total deaths at third stimulation (3 weeks).

(Analysis of Dunnett test yields similar results to Fisher and Scheffe)

One Factor ANOVA X_1: Inducibility Y_1: Infarct sizes

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	188.059	94.03	7.586
Within groups	54	669.36	12.396	p = .0012
Total	56	857.419		

Model II estimate of between component variance = 40.817

One Factor ANOVA X_1: Inducibility Y_1: Infarct sizes

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Group 1	12	11.092	5.06	1.461
Group 2	39	7.015	2.878	.461
Group 3	6	5.4	3.822	1.56

One Factor ANOVA X_1: Inducibility Y_1: Infarct sizes

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Group 1 vs. 2	4.076	2.33*	6.15*	3.507
Group 1 vs. 3	5.692	3.53*	5.227*	3.233
Group 2 vs. 3	1.615	3.096	.547	1.046

* Significant at 95%

Table 10.4 (legend on following page)

Table 10.4: Analysis of the relationship between inducibility and infarct size (One-Way Analysis of Variance with Multiple Comparisons).

Key: Group 1, Sudden deaths within first week.

Group 2, Inducible dogs assessed at first stimulation (1 week).

Group 3, Non-inducible dogs.

(Analysis of Dunnett test yields similar results to Fisher and Scheffe)

Chapter 11. DISCUSSION

The experiments described in this thesis raise several interesting points with regard to the aims stated earlier. Firstly, attention has been drawn to the role of adrenergic factors in the genesis of ventricular arrhythmias, and in particular how the respective contributions of α and β stimulation may vary under conditions of ischaemia and infarction. Secondly, it has been shown that protection against malignant ventricular arrhythmias and sudden death may not necessarily be anticipated from a drug's known electrophysiological properties in vitro. We may have helped clear some of the controversy surrounding the use of H_2 receptor antagonists in patients with ischaemic heart disease, but we have shown that an established antiarrhythmic agent may possess life-threatening proarrhythmic features. Finally, valuable information is provided with regard to the applicability of post-infarcted canine models for chronic electrophysiological studies. These points are considered in turn below.

11.1 Adrenergic Factors in Arrhythmogenesis

Despite Govier's description of myocardial α receptors over 20 years ago, studies into the cardiac effects of adrenergic stimulation continued to concentrate largely on the β adrenergic system, possibly because the physiological effects of myocardial α stimulation remained unclear. Interest however was renewed after the observation that a number of α adrenoceptor antagonists

exhibited antiarrhythmic effects in experimental models. A further step was the classification of α adrenoceptors into α_1 and α_2 subtypes (Langer, 1977); this enabled further exploitation of the antiarryhythmic potential of α_1 adrenoceptor antagonists without the attendant risks of increased myocardial noradrenaline concentrations seen with non-specific α blockade. Results with α adrenoceptor antagonists, however, have always been open to the argument that any antiarrhythmic activity may be secondary to peripheral (including coronary artery) α antagonism. Accordingly, UK-52046 has been developed as an α_1 adrenoceptor antagonist with selective affinity for myocardial α receptors. Radioligand binding studies have confirmed marked affinity for post-junctional α_1 adrenoceptors ($K = 4.5 \times 10^{-11}$) and preliminary results have suggested antiarrhythmic activity without the haemodynamic consequences of peripheral vasodilatation (Pfizer Central Research, unpublished communication).

Results of our studies confirm the antiarrhythmic potential of the drug: thus in the halothane-adrenaline model UK-52046 was effective at a dose of 3.8 ± 1.4 ug/kg while a comparable protective dose of atenolol was 14.6 ± 2.1 ug/kg. This is similar to the results of Maze and Smith (1983) with the α_1 adrenergic antagonist prazosin, and it is interesting that we, too, demonstrated a synergistic response with a combination of α and β antagonism (effective dose of each 0.36 ± 0.1 ug/kg). UK-52046 did not produce

a significant fall in blood pressure in this experiment, but the pressor response to adrenaline was attenuated: in view of what has already been said regarding the factors necessary for the production of the arrhythmia (chapter 3.2.2) it might therefore be argued that the drug was antiarrhythmic by nature of a peripheral haemodynamic action. In a modification of the same experiment which does not depend on a pressor response, however, the parent company has infused adrenaline into a halothane-respired anaesthetised dog at such a rate as to produce a continuous ventricular arrhythmia. In these experiments the addition of increasing intravenous doses of UK-52046 has resulted in abolition of the arrhythmia without accompanying falls in blood pressure (Pfizer Central Research, unpublished communication).

Significant falls in blood pressure were however apparent in the arrhythmia occurring 24 hours after coronary artery ligation, where a significant increase in the number of sinus beats resulted from the administration of 16 mg/kg of drug. Earlier studies with non-specific α antagonists had shown little effect on this particular arrhythmia (Maling et al, 1959), but more recent investigations with prazosin using isolated papillary muscle and Purkinje fibre preparations have shown a depression in the maximal upstroke velocity and a dose-dependent prolongation of action potential duration (Dukes and Vaughan Williams, 1984). It is possible that UK-52046 may share some of these electrophysiological effects and possess sufficient class 1 activity to suppress the '24 hour'

arrhythmia while at the same time being unable to influence the ouabain-induced arrhythmia. It is of interest that atenolol exacerbated the ouabain-induced arrhythmia, since reference has already been made to this phenomenon with certain alternative β adrenergic antagonists (chapter 3.2.1).

One particularly interesting aspect to the studies described in chapter 5 was the finding that, while atenolol (100 ug/kg) significantly reduced the ectopic response to adrenaline after coronary artery ligation, it was less effective than UK-52046, where a dose of 3.7 ± 1.4 ug/kg prevented the arrhythmia in 6/6 dogs. Furthermore, the combination of α_1 and β_1 antagonism appeared no better than α_1 blockade alone. While this may be at variance with the synergy observed in the halothane-adrenaline model, it may be seen as a confirmation of the enhanced α responsiveness following ischaemia (Juhasz-Nagy and Aviado, 1976; Sheridan et al, 1980). In this model too, haemodynamic changes were apparent at higher doses of UK-52046, but as in the '24 hour' model, this was a feature only at doses greater than those at which antiarrhythmic efficacy was apparent. It is presumably the intact autonomic reflexes in the conscious animal which mediates the tachycardia in the adrenaline-induced arrhythmia after coronary artery ligation, since this (the tachycardia) was not a feature in the halothane adrenaline model.

A dose-dependent hypotensive action was also apparent in the arrhythmias of acute coronary ischaemia, where we demonstrated a

significant antiarrhythmic effect of UK-52046 at a dose of 8 ug/kg. These results are in agreement with similar studies using various α adrenoceptor antagonists in the dog (Benfey et al, 1984), the guinea-pig (Penny and Sheridan, 1982), the cat (Sheridan et al, 1980; Davey, 1981) and the pig (Benfey et al, 1984). Having demonstrated this effect in one reentrant model, it is interesting to speculate on why the drug was ineffective against those arrhythmias generated by programmed electrical stimulation. This may relate to the severity of the particular model used: we have already seen from chapters 7 and 8 that mexiletine and racemic tocainide respectively failed to demonstrate significant protection in the same model despite apparent efficacy in alternative arrhythmia models. The use of conscious dogs is a further factor which must be taken into account, as it allows the study of potential antiarrhythmic action where the arrhythmogenic conditions of high circulating catecholamines and enhanced sympathetic tone are not blunted by general anaesthesia. Our results with an α_1 adrenoceptor antagonist and programmed stimulation are similar to those of Wilber et al (1987) who demonstrated no protection against the arrhythmias of programmed stimulation in conscious dogs dosed with prazosin. In the same study, however, prazosin afforded significant protection against the arrhythmias of acute ischaemia, in this case ischaemia produced by the introduction of a 150 uA current in the left circumflex coronary artery (chapter 3.3.3). Since, in the study mentioned, prazosin (like UK-52046 in our study) did not produce any

electrophysiological changes, the authors conclude "the antifibrillatory efficacy of (prazosin) is not derived from direct electrophysiologic actions manifest in normal noninjured tissue, but rather possibly is due to primary or secondary electrophysiologic actions displayed in acutely ischemic tissue or to a blunting of those electrophysiologic consequences of acute ischemia that are mediated by α_1-adrenergic stimulation". In support of the latter hypothesis, the authors point to the work of Sheridan and Culling (1985) which showed that the attenuation of the electrophysiological consequences of ischaemia by α adrenergic antagonists was mediated by adrenergic rather than direct myocardial electrophysiological effects in an isolated preparation.

Our results did not demonstrate any significant electrophysiological changes after UK-52046, but previous studies in isolated preparations have demonstrated α-mediated changes in refractory periods (Govier, 1967), positive inotropy (Schumann, 1978) and prolongation of action potential duration (APD) (Benfey, 1982). If, as has been suggested, α adrenergic responsiveness is enhanced under ischaemic conditions, then α-mediated prolongation of APD in ischaemic areas may combine with β-mediated shortening of APD in non-ischaemic areas to produce the arrhythmogenic milieu suitable for the development of reentrant pathways (Vaughan Williams, 1985). The cellular events mediating these changes remain unclear, as contractility appears to be augmented without increases in cyclic AMP (unlike β stimulation) (Watanabe, 1977); nevertheless,

it is thought that these α-mediated effects may involve modulation of intracellular calcium (Sharma et al, 1983). Intracellular calcium accumulation during myocardial ischaemia is known to contribute to the disruption of cellular metabolism and to the production of irreversible injury (Nayler et al, 1979; Jennings and Reimer,1981).

Our studies have demonstrated significant haemodynamic changes after administration of UK-52046. It is a limitation therefore of all the models, but the arrhythmias of acute coronary ischaemia in particular, that we were not able to include in our experimental protocol the measurement of either coronary artery or regional myocardial blood flow. Alpha adrenergic receptor stimulation is thought to play an important role in the regulation of coronary blood flow (Thomas et al, 1984), but studies have suggested that the effects of catecholamines on coronary resistance may be mediated by α_2 receptors (Holtz et al, 1982; Heusch et al, 1984) and that collateral coronary vessels may be devoid of α receptors in general (Murray et al, 1984). Furthermore, in their study of α adrenergic contributions to dysrhythmias during myocardial ischaemia and reperfusion in cats Sheridan and coworkers (1980) studied regional myocardial blood flow with radiolabelled microspheres and found that pretreatment with the non-specific α antagonist phentolamine had no effect on flow during any of the intervals studied.

Few results are available for studies of α adrenoceptor antagonists in man: Nickerson and Brown (1951) showed that

pretreatment with the non-specific α antagonist dibenamine resulted in an almost complete suppression of the arrhythmias associated with cyclopropane anaesthesia. Antani and Srinivas (1973) in a study of 25 patients, infused phentolamine intravenously at a rate of 0.5 mg/min: of 13 patients with ventricular arrhythmias of various aetiologies, 10 (77%) responded with complete abolition of the arrhythmia while the remaining 3 (23%) exhibited partial suppression. Gould et al (1975) studied the effects of oral phentolamine, 50 mg four times daily, in a study of 30 patients with uncomplicated acute myocardial infarction: in a double-blind, placebo-controlled study they demonstrated a significant ($p<0.05$) reduction in the number of ventricular ectopic beats in the patients receiving phentolamine. Our results suggest that α_1 adrenergic modification may prove to be a protective therapeutic measure in patients with recurrent ischaemic attacks, a population known to be at high risk of sudden death.

11.2 Drug Protection Against Ventricular Tachycardia and Death

Our studies with programmed electrical stimulation in a chronic canine model of myocardial infarction have shown significant antiarrhythmic protection for R and S tocainide and atenolol; conversely, the use of racemic tocainide and mexiletine did not reach statistical significance when compared with placebo.

With regard first to the results of our study with the enantiomers of tocainide, it has been recognised for some time that

the separation of the optical isomers of antiarrhythmic compounds may be associated with differentiation of specific actions (Howe, 1963; Howe and Shanks, 1966). Much of the initial work in this field has concentrated on the β adrenoceptor antagonists (Whitsitt and Lucchesi, 1967; Howitt et al, 1968; Barrett and Cullum, 1968), but more recently studies have been extended to include also the class 3 (Lynch et al, 1984) and class 4 (Bayer et al, 1975; Au et al, 1987) agents. In the group of dogs which received racemic tocainide, 3 became non-inducible (not significant when compared with placebo). This is similar to a previous study using programmed stimulation in post-infarcted dogs, where 4 of 8 animals became non-inducible after the racemic mixture (Brachmann et al, 1984). A corresponding clinical study (Easley et al, 1985) demonstrated only 10-20% efficacy with the drug. There are no reports of specific antiarrhythmic studies with the enantiomers of tocainide, but Woosley and coworkers in Nashville are reported to have demonstrated a three-fold affinity for the sodium channel when the R enantiomer was compared with the racemic mixture. (personal communication). Tocainide has been reported to exacerbate certain arrhythmic states, both in animal (Brachmann et al, 1984) and clinical (Velebit et al, 1982) studies, but there was no suggestion of a proarrhythmic response to the racemate or either enantiomer in our studies with the drugs.

Mention has already been made of the ability of class 1b drugs to shorten action potential duration (APD) in in vitro studies using

intracellular micro-electrodes (chapter 2.2.2). However, the same drugs also slow the kinetics of the sodium channel, so that the effective refractory period (ERP) of the tissue is not shortened to the same extent as the APD and the ERP:APD ratio is effectively increased (table 2.2). In the present study with the drug in vivo, the QT_C interval (as an index of APD) showed no significant change as the result of racemate or either enantiomer and refractory periods were similarly unchanged (figure 8.3). Our results are in agreement with earlier in vivo studies which showed that tocainide does not alter AV conduction (PR interval) or intraventricular conduction (QRS duration) (Anderson et al, 1978; Moore et al, 1978; Oshita et al, 1980). In vitro studies, however, have demonstrated enantiomeric differences in maximal rates of depolarization indicating a specific receptor interaction and a possible explanation for the differences in antiarrhythmic activity (Carmeliet et al, 1986; Almgren et al, 1987). Our finding of pharmacological differences between the R(-) enantiomer and the racemic compound in this class 1 antiarrhythmic agent must now open up the field of conventional antiarrhythmic therapy, with attention being focused on active moieties within parent compounds. It is also possible that some of the side effects of tocainide, such as those which have restricted the use of the drug in the UK (Volosin et al, 1985) may be related to enantiomeric characteristics.

Mexiletine was a further class 1b drug whose effects were compared with those of atenolol in the study described in chapter 7

and whose enantiomers were the subject of investigation in chapter 8. Like racemic tocainide, mexiletine was not found to be significantly different from placebo in protecting against the arrhythmias of programmed stimulation. While there appears to have been little work with animal models, the effects of racemic mexiletine on the inducibility of arrhythmias by programmed electrical stimulation in man has been variously reported by a number of authors (Table 11.1).

Investigator	No. of Patients	Monotherapy (%)	Combination (%)
Ruskin et al, 1980	25	9 (36)	13 (52)
Dimarco et al, 1981	35	9 (26)	13 (37)
Breithardt et al, 1981	12	4 (33)	9 (75)
Palileo et al, 1982	11	1 (9)	--
Podrid et al, 1983	36	3 (8)	11 (31)
Waspe et al, 1983	33	1 (3)	7 (21)
Saksena & Craelius, 1983	19	7 (37)	--
Kasanuki et al, 1983	20	8 (40)	--
Fenster and Kern, 1983	5	2 (40)	--
Schoenfeld et al, 1984	118/148	23/188 (19)	53/148 (36)
Kim et al, 1986	25	--	4 (16)

Table 11.1

Previous results from the use of mexiletine in electrophysiological studies with programmed electrical stimulation. Values include examples of where mexiletine was tested alone (monotherapy) and in combination with various other antiarrhythmic agents (combination).

By far the largest study to date has been that of Ruskin and coworkers (1980); using a study population of 148 patients they were able to induce ventricular tachyarrhythmias in all 148 in the absence of antiarrhythmic drugs. After the baseline electrophysiological evaluations all patients then underwent serial drug testing with one or more conventional antiarrhythmic agents: of 118 patients tested while taking mexiletine alone, 23 (19%) showed complete suppression of inducible tachyarrhythmias; in addition, complete suppression was also observed in a further 30 patients tested with mexiletine in combination with one or more alternative antiarrhythmic agents (overall suppression 53/148 or 36%). Dimarco et al (1981) studied 35 patients with electrically inducible ventricular arrhythmis, all of which were resistant to conventional therapy. Mexiletine, either alone or in combination with a previously ineffective agent, resulted in complete suppression of inducible arrhythmias in 13 patients; in a further 7 the response to stimulation was said to be 'favourably modified'. In general, results of the use of mexiletine alone range from 3% to 40% (Table 11.1). Such figures, however, should be viewed in the light of disagreement over standard pacing methods and stimulation protocols; definitions of sustained and non-sustained ventricular tachycardia vary between authors as do end-points for drug response (complete suppression of arrhythmia, conversion of sustained into non-sustained tachycardia or ability to reproduce arrhythmis only with more aggressive stimulation). Again like our results with tocainide, we demonstrated

no change in any electrophysiological parameter when mexiletine or either enantiomer was compared with placebo. This is in agreement with previous studies which have shown no effect on action potential duration (Singh and Vaughan Williams, 1972), conduction times (Okuma et al., 1976) or refractory periods (Campbell, 1982). In the present study, 16 mg/kg of mexiletine produced a significant tachycardic effect. Similar effects have previously been ascribed to a vagal inhibitory action (Touboul et al., 1978) but this would seem unlikely without associated delays in conductivity, in particular atrioventricular times (as reflected in PR intervals). Since the increase in heart rate was only apparent after 16 mg/kg, a dose which produced invariable features of agitation and rigidity, it would seem more likely that this finding was a reflection of drug toxicity. To the best of our knowledge, our studies with the enantiomers of mexiletine represent the first such experiments with these isomers. R and S mexiletine have been the subject of pharmacokinetic studies (Grech-Belanger et al, 1986), but there has not been an evaluation of pharmacological effect.

In comparison to our results with mexiletine, the β_1 specific adrenoceptor antagonist atenolol exhibited significant anti-arrhythmic activity in our study with programmed stimulation. This is similar to previous electrophysiological studies in chronic canine models which have demonstrated significant antiarrhythmic and antifibrillatory effects propanolol (Echt et al, 1983), timolol (Gang et al, 1984) and sotalol (Patterson et al, 1984). Experience with

programmed electrical stimulation in man appears limited and unclear; Kasanuki et al (1983) found propranolol to be ineffective in each of 3 patients under study, while Kim et al (1986) found the same drug effective (alone and in combination) in preventing the induction of ventricular tachycardia in 10 of 25 patients.

It has been suggested that studies such as these are more appropriate as evidence of the antifibrillatory effects of β adrenergic blockade in the heart, but our study clearly shows that atenolol prevented the recurrence of ventricular tachycardia as well as protecting against ventricular fibrillation. These antiarrhythmic studies are similar to those of Rossi et al (1983) who, in a study of 182 patients given intravenous atenolol within 5 hours (mean) of the onset of chest pain, found a threefold ($p<0.001$) reduction in the incidence of ventricular ectopic beats.

Unlike many β adrenergic receptors antagonists, atenolol is devoid of the membrane-stabilising properties which have been thought responsible for antiarrhythmic effects of beta-blockade in the past (Somani and Lum, 1965). Nevertheless, we did identify 2 electrophysiological characteristics which did distinguish atenolol from mexiletine and placebo, and which may account for the drug's antiarrhythmic action. Prolongation of the effective and functional refractory periods has been noted previously (Gang et al., 1984) and is most likely a reflection of antagonism of the high levels of circulating catecholamines and sympathetic tone pertaining in the conscious dogs during programmed stimulation: both sympathetic

nerve stimulation (Kralios et al., 1975) and exogenous catecholamine administration (Hoffman and Singer, 1967) have been shown to shorten refractory periods but beta-adrenoceptor antagonism has been shown to have no effect on refractoriness in anaesthetised dogs (Hoffman and Singer, 1967). Nevertheless, by delaying depolarisation of myocardial fibres in possible reentrant pathways, this antagonism of catecholamines-induced reduction in refractoriness must be considered protective. The significant fall in heart rate observed in the study may be similarly antiarrhythmic; El-Sherif and coworkers (1978), in a study of reentrant ventricular arrhythmias in the late myocardial infarction period in the dog demonstrated that the propensity of sympathetic stimulation to induce arrhythmias was primarily due to its tachycardic effect. A previous study with propranolol (Hope et al., 1974) had shown a protective action of propranolol which was antagonised when the heart rate was returned to control values by atrial pacing. Furthermore, in a review of some of the larger prospective trials with the β adrenergic antagonists, Kjershus (1985) drew attention to the almost linear relationship between the reduction in resting heart rate and the associated fall in mortality (figure 11.1).

11.3 Proarrhythmic Effects of Drugs

Results from chapter 9 indicate that neither cimetidine nor ranitidine are proarrhythmic in a chronic canine model, the suitability of which was confirmed by the proarrhythmic effects of

quinidine.

The first description of chronic canine models being used for proarrhythmic studies appears to have been in 1980 when Lucchesi and coworkers, in a study of the antiarrhythmic effects of procainamide, showed that ventricular tachycardias could be exacerbated by subtherapeutic levels of the drug. These findings led to a study (Patterson et al, 1981) where non-inducible dogs were treated with intravenous lignocaine before being rechallenged with programmed stimulation. Of the 16 animals tested, repeat stimulation produced non-sustained VT in 4, sustained VT in 9 and ventricular fibrillation in 1. Similar results have been demonstrated in canine models with the class 1c drug flecainide (Zimmermann et al, 1985; DiCarlo et al. 1985). There appears to have been very little experimental work involving other (non-antiarrhythmic) drugs, but Dobmeyer et al (1982) demonstrated alterations in refractory periods associated with proarrhythmic responses in 8 of 9 patients following the administratiuon of 200 mg of caffeine.

It has already been mentioned how inhomogenous prolongation of the QT interval may be an inherently proarrhythmic mechanism (chapter 3.4), and this is a possible explanation for our results with quinidine, since QT_C increased from 0.325 ± 0.01 to 0.355 ± 0.005 ($p<0.05$ compared with placebo and pretreatment values). It is interesting that the ERP did not alter as the result of drug, since this represents an effective decrease in the ERP:APD ratio (table 2.2). The anticholinergic effects of the drug may also be a factor

(Goldstein et al, 1973) and drug levels too may be important, since many antiarrhythmic drugs exhibit a biphasic response curve with reversal of antiarrhythmic effects at higher doses. This has been suggested for quinidine (Heissenbuttel and Bigger, 1970), although proarrhythmic effects have been demonstrated within the usually accepted 'therapeutic' range (Jenzer and Hagemeijer, 1976).

No explanation for a possible proarrhythmic action of the H_2-receptor antagonists has been proposed, but it has been suggested that by blocking dopamine receptors, the drugs may facilitate arrhythmia production by virtue of the associated hyperprolactinaemia (Cohen et al, 1979). It is known that cimetidine therapy is associated with an elevated serum prolactin level (Carlson and Ippoliti, 1977); furthermore, prolactin is known to be proarrhythmic in rats (Nassar et al, 1974) and patients with prolactinomas may exhibit unexplained arrhythmias (Cohen et al, 1979). Since many of the patients to whom H_2 antagonists are administered intravenously are frequently seriously ill, it may be argued that any association with life-threatening arrhythmias is a reflaction of the underlying disorder. It is also possible that the arrhythmias are related to the high histamine levels often seen in such patients (Cohen et al, 1979; Watson et al, 1982), since recent reports suggest an association between ventricular arrhythmias and the use of H_1 antagonists (Craft, 1986).

The study showed no change in QT_c, PR or QRS as the result of

treatment with cimetidine or ranitidine. These results are similar to those of Gould et al (1981) who detected no change in AV conduction or QRS duration in patients given cimetidine. Since these parameters are all derived from conduction charateristics, our results are in agreement with Levi et al (1976) who suggested that histamine's effects on conduction were mediated by H_1 receptors. If cimetidine and ranitidine were to shorten refractory periods, a proarrhythmic effect could be explained on the basis of early depolarization of adjacent myocardial fibres in a reentrant pathway. While we did demonstrate some shortening of both the ERP and FRP, with cimetidine these changes did not reach significance and with ranitidine numbers were too small to permit statistical analysis.

All the drugs under study produced significant increases in heart rate when compared with placebo. Anticholinergic effects are most likely the cause of this phenomenon with quinidine, but it is less apparent why a tachycardia should be observed with cimetidine or ranitidine. Bradycardia, however, has not been a universal finding with these drugs. Engel and Luck (1979), in a study of 10 patients before and after an infusion of 300 mg of cimetidine, were unable to demonstrate any significant effect on sinus node function, and toxicological studies in dogs have reported tachycardia in association with high-dose cimetidine therapy (Leslie and Walker, 1977). In view of the doses at which tachycardia became apparent in this study (cumulative doses of 32 mg/kg and 16 mg/kg for cimetidine and ranitidine respectively) it seems most likely that

this has been a feature of drug toxicity in both cases.

When considering the H_2 antagonists in the field of cardiac disease in general, it is interesting to speculate on a recent article (Toda, 1987) which demonstrated that histamine-induced coronary artery spasm was mediated by H_1 receptors in smooth muscle, and that coronary artery dilatation was a feature of H_2 stimulation. One conclusion of this might be that the use of H_2 receptor antagonists could produce unopposed H_1-mediated coronary artery spasm and, if what we have said regarding the patho- physiological aetiology of sudden cardiac death is true, this might be a contributory factor, especially in the post-infarction population. A personal communication from the Boston Collaborative Drug Surveillance Program in Massachusetts, however indicated that this problem had not as yet been addressed in man.

11.4 The Significance of Infarct Sizes

Our results from chapter 10 confirm a reduction in the inducibility of arrhythmias as a function of time following experimental myocardial infarction. This would appear to be the only such study, for although anecdotal stories exist of dogs exhibiting arrhythmias on stimulation for up to 2 years and more, we have been unable to find any evidence of this in the literature, nor would such stories be in keeping with our results.

One argument which may be levelled against our findings is that

the most inducible animals will die earlier as the result of programmed stimulation; thus any evaluation of inducibility against time will have an inherent bias. To overcome this particular problem would require a study where only surviving animals were included for statistical analysis. Another possibility, however, is that the reduction in inducibility reflects a reduction in infarct size. It is known clinically that scar formation usually becomes apparent during the third week after myocardial infarction. Increased fibroblastic activity associated with removal of necrotic tissue and the appearance of widening bundles of collagen ultimately results in a thin scar, formed principally from pre-existng stroma and new connective tissue (Hurst et al, 1982). Our results show a tendency towards a time-dependent reduction in infarct size, although some inter-group variations in the later weeks did not reach statistical significance. Using a similar chronic canine model, Wilber and coworkers investigated this phenomenon in a study of 30 dogs subjected to programmed electrical stimulation 5 days after coronary artery ligation. Results indicated that infarct size was much larger in inducible (24.7 ± 1.7% of LV mass) than in non-inducible (5.3 ± 1.1%) ($p<0.001$) animals (Wilber et al, 1985).

One particularly interesting aspect to this study was the observation that a number of previously well animals died suddenly and unexpectedly during the first postoperative week. That fact that 2 of these dogs died when exercised outside in cold weather suggests that coronary vasospasm may have been involved:

ventricular electrical instability is known to be at a maximum until the sixth post-operative day in the dog (Thompson and Lown, 1972) and a similar model of distant ischaemia has been successfully employed for sudden death studies (chapter 3.3.3). Taken as a group, these animals had a significantly greater mean infarct size than surviving dogs, either inducible or non-inducible. In the same analysis, the difference between inducible and non-inducible animals did not reach statistical significance (table 10.4). However, these results are heavily influenced by the nature of weekly stimulations for the dogs under study: to study more closely the phenomenon of infarct size as a function of inducibility would require sacrificing every animal when first stimulated.

In general, the infarct sizes obtained in our studies are smaller than those published by other investigators using mongrel dogs. This may well relate to the nature of the animal under study; collateral circulations are known to vary greatly amongst different species of dog and may have an important role in determining the outcome of coronary ligation (Chardack et al, 1964). Sudden occlusion of the left anterior descending artery in beagles has been shown to produce little ischaemia (as assessed by early arrhythmic activity) (Shanks and Dunlop, 1967), while in greyhounds a critical stenosis alone produces sufficient ischaemia to be included as an arrhythmia model (chapter 6). Many of the animals which died from acute ischaemic ventricular fibrillation during surgery were those in which attempts had been made to produce larger infarcts; however, since results of

staining with TTC were rarely successful when attempted less than 2 hours from the initial ligation, this is difficult to assess scientifically.

Macroscopic enzyme-mapping verification of infarct size using TTC appears to be an accurate method of determining the extent of experimental myocardial necrosis (Lie et al, 1975; Fishbien et al, 1981). Although it has been suggested that the method may not accurately predict recovery of myocardial fibres after reperfusion (Barnard et al, 1986), it nevertheless remains the investigation of choice in both occlusion/reperfusion (Simpson et al, 1987) and occlusion-only (DeBoer et al, 1982) studies. In our studies, infarct mass was determined gravimetrically; computer-assisted planometric determination of infarct mass has also been described, but there does not appear to be any distinct advantage between the two methods (Lynch et al, unpublished results).

11.5 Criticisms, Suggestions and Future Directions

I include this section primarily to concentrate on certain specific aspects of our work which may be considered controversial, or for which (during the course of manuscript review) we have been criticised. Automaticity models of cardiac arrhythmias have always been open to questions regarding their applicability to the clinical situation and I do not intend to add further to this particular debate; instead I would wish to limit this section specifically to some of the arguments relating to programmed electrical stimulation in the

chronic canine model.

One particular criticism of our model relates to the finding that the use of 3 closely-coupled extrastimuli may result in arrhythmias in normal dogs (Hamer et al, 1984). While we accept these results, we would point out that in the particular study referenced, the end-point for arrhythmia induction was ventricular fibrillation, and the technique employed stimulating current strengths of up to fifteen times diastolic threshold. It is well known that currents of sufficient strength will produce fibrillation (such is the basis for the ventricular fibrillation threshold) and that threshold currents are reduced with increasing numbers of extrastimuli (Moore et al, 1986), but this is the reason why we have consistently stimulated at twice diastolic threshold. We would also note the results of several previous studies which have failed to produce arrhythmias using similar pacing methods in sham-operated dogs (Michelson et al, 1981; Garan et al, 1981; Patterson et al, 1982(b); Lown et al, 1986). When ventricular fibrillation did occur during stimulation, it was frequently preceeded by a variable period of sustained monomorphic ventricular tachycardia (figures 11.2, 11.3) and in this respect parallels the findings for sudden cardiac death in man (chapter 1.3.2).

Animals which fibrillated during the course of our studies were not resuscitated for two reasons: initial experience with standard defibrillation equipment had been singularly ineffective in reversing fibrillation, and even with modified plates strapped to the animals'

chests we were able successfully to defibrillate only a minority of dogs. Another reason for not pursuing the problem was to avoid the confounding influence of repeated and occasionally prolonged resuscitative efforts on the outcome of subsequent investigations (Ehsani et al, 1976). Since the survival of the animals was a major consideration in all of the studies with programmed stimulation, this was one reason for not pursuing a reproducible non-sustained VT with more aggressive pacing methods in an attempt to achieve a sustained arrhythmia. Our experience has been that increasing the nunber of extrastimuli and reducing coupling intervals may result in a higher incidence of sustained VT, but at the expense of greater pretreatment mortality. In this respect our results are similar to the experiences of Lucchesi and co-workers (Lynch et al, 1987). Futhermore, results from the same laboratory have shown a highly predictive relationship between the vulnerability to initiation of *either* sustained *or* non-sustained VT (by programmed stimulation) to the development of sudden death in response to a subsequent ischaemic insult at a distant site (chapter 3.3.3)(Wilber et al, 1985), thus confirming non-sustained VT as an adequate substrate from which to base an antiarrhythmic study. It has been argued that unimorphic VT is more specific for ischaemic damage than polymorphic arrhythmias. While this may be proven for clinical electrophysiological testing, the question does not appear to have been addressed in animal studies. For this reason we accepted both, providing the non-sustained arrhythmias were reproducible in any

given animal.

Resuscitation of animals might have been a more straightforward procedure had we chosen to use anaesthetised, open-chested animals for programmed stimulation. While such a method would also have made more simple the management of arterial access and the occasionally agitated animal, it would have restricted the number of times we could have stimulated any particular dog. Furthermore, we believe the relevance of a conscious model is of crucial importance in the evaluation of antiarrhythmic agents, especially when dealing with drugs with inherent sympatholytic activity such as the α or β-adrenergic antagonists. The role of an intact autonomic nervous system in the genesis of cardiac arrhythmias has been recognized for some time (Lown and Verrier, 1976; Corr and Gillis, 1978), and in a similar chronic canine model, Schwartz and Stone (1980) have demonstrated that sympathetic stimulation can facilitate the induction of ventricular fibrillation; furthermore, neurogenic activity can modify the effects of antiarrhythmic drugs (Bigger et al., 1982).

Our definition of non-sustained VT was reached arbitrarily. Since this field of experimental pharmacology is relatively new, there has been little time for agreement on standard definitions for arrhythmias; thus the recent Lambeth conventions are particularly welcome in their recognition and tackling of such problems, even if decisions may not have met with unanimous approval (Walker et al, 1987). Based on the studies of Gibson and Lucchesi (1980),

[non-sustained VT as 3 or more ventricular ectopic beats]; Michelson et al (1980(b)), [3 or more]; Lynch et al (1984), [5 or more]; Cobbe et al (1985), [4 or more] and Moore et al (1986), [4 or more], we chose 4 ventricular ectopic beats as the minimum requirement for establishing a non-sustained VT. In retrospect we are able to stand over this figure, since once an animal exhibited 4 extra beats, we could be fairly sure that, on rechallenge, this would be at least consistent, if not associated with a more dramatic ectopic response. Conversely, non-inducible dogs commonly produced 2 or 3 extra beats during pre-drug stimulation, but only one of the 24 animals studied developed 4 extrasystoles, and this only once during the protocol.

In retrospect, our definition of sustained VT was probably too demanding. Again, we were faced with conflicting figures from other researchers and the production of prolonged periods of VT in some early studies led us to adopt the criteria of a self-perpetuating arrhythmia of 5 min duration as our standard. While there can be no argument as to the authenticity of this as a sustained arrhythmia, in retrospect it was too severe a model, and led to the loss of a number of dogs which fibrillated after a variable period of arrhythmia. This probably relates to haemodynamic compromise, and is the reason for termination of the arrhythmia by burst pacing by a number of other researchers (Michelson et al, 1980(a); Garan et al 1980; Lynch et al, 1984; Cobbe et al, 1985). Thus it might be prudent in future studies to refine our definition of sustained VT to 'a self-perpetuating

arrhythmia of 30 sec duration or which required overdrive pacing for termination'. In this way we would avoid the high mortality from pretreatment stimulation (chapter 10).

Reference has already been made to the theoretical advantage of using an occlusion/reperfusion model in studies with programmed electrical stimulation (chapter 3.3.2). Not having compared the two methods in a scientific study, we are not in any position to comment on this particular debate, but we can dispel the argument that reperfusion is an essential prerequisite for arrhythmia generation (Karagueuzian et al, 1979). Whether this may be a feature of our particular model (the greyhound) we cannot say, but it is clear that by occluding alone we avoid the high incidence of reperfusion ventricular fibrillation (Battle et al, 1974).

Our electrophysiological studies included the measurements of electrocardiographic parameters and refractory periods. Possible modifications here might include the separate determination of infarct and normal zone refractoriness, possibly by constructing strength/interval curves along the lines first suggested by Michelson et al (1980(a)). This would obviously involve adaptation of the unipolar electrodes presently used, with conversion also of threshold voltages to amperage values, but would allow the study of drug effects in both infarcted and normal myocardium. Ventricular activation times and VT cycle lengths are essentially measures of conductivity and can be determined from normal and infarcted electrograms and the surface electrocardiogram respectively (Lynch

et al, 1987). Using the equipment presently employed, there is no reason why these parameters should not be added to those variables currently measured. One final point relates to the measurement of the QT interval. Reference has already been made to Bazett's discovery of an inverse relationship between the QT interval and heart rate, but although the equation is well known (and widely used), there are those who remain unconvinced of the accuracy of the calculation (Somberg et al, 1985). For this reason, many electrophysiologists now refer to the paced QT, a QT interval as determined during fixed atrial pacing. The inability of our particular model to deal with variations in coronary artery or regional myocardial blood flow has been considered in detail already (chapter 11.1).

And future directions? As regards surgical proceedures or stimulation protocols, the inclusion of additional atrial and normal zone/ischaemic zone electrodes could provide further electrophysiological information, while chronically implanted arterial and venous lines (for example external jugular and carotid catheters exteriorized at the nape of the neck) might allow easier access for blood pressure recording and administration of drugs.

Much has been done in relating induction of arrhythmias with programmed stimulation to the site of stimulation, stimulus duration, intensity and polarity, the basic stimulation rate and the number of extrastimuli (Moore et al, 1986). There appears to have been little research, however, into the effects of variations in those

basic biochemical parameters which may be of crucial importance under ischaemic conditions (chapter 1.1.5). Thus a final refinement of our experimental set-up might be to monitor arterial oxygen tension, pH, and electrolyte status, and in so doing note any associated alterations in inducibility of arrhythmias.

The field of antiarrhythmic drug therapy remains one of the most fascinating and challenging facets of medicine today, and I believe our studies open up a number of possible research projects for the future. Much has still to be learned about adrenergic mechanisms in normal and infarcted myocardium, while the protection afforded by the β–adrenergic antagonists in programmed stimulation could be followed by studies which concentrate on slowing of heart rate (for example with the specific bradycardic agents) or refractoriness (for example with the class 3 agents). Our results with the enantiomers of tocainide should hopefully stimulate interest into isolating and testing the isomers of other antiarrhythmic agents. Proarrhythmic studies are in their infancy, and must become a routine investigation in the 'work-up' of new antiarrhythmic agents as well as a host of drugs with direct or potential effects on the heart.

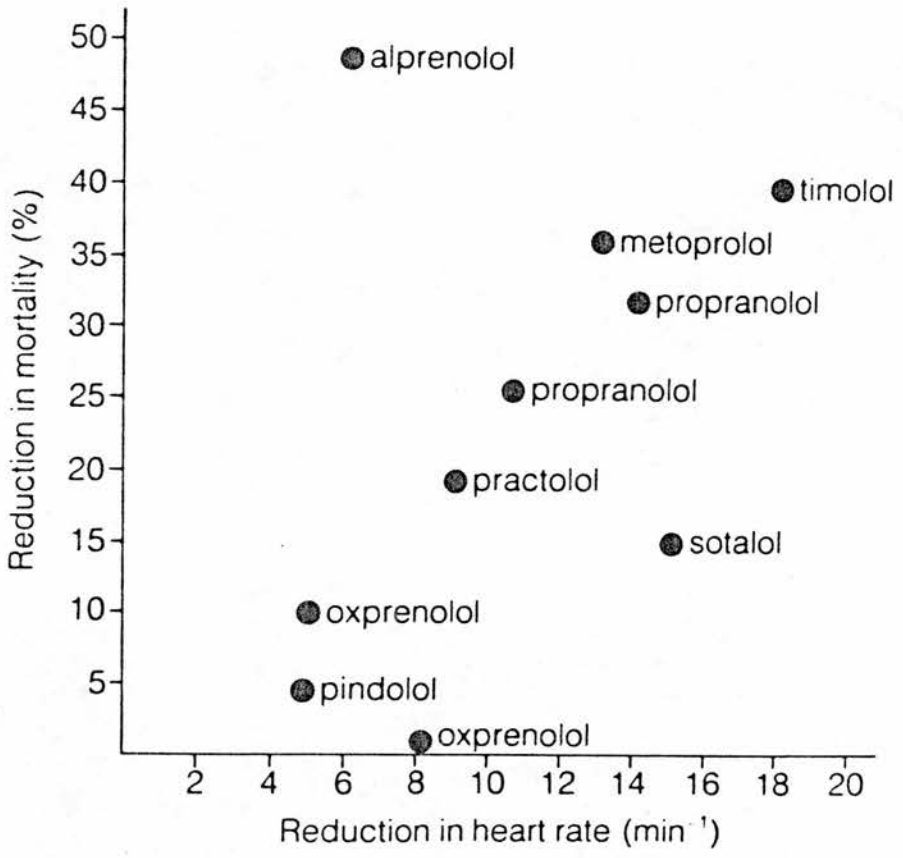


Figure 1.

Relationship between fall in heart rate and reduction in mortality using some of the commoner beta-adrenergic receptor antagonists: review of several prospective trials.

(Reproduced from: Kjekshus,1985)

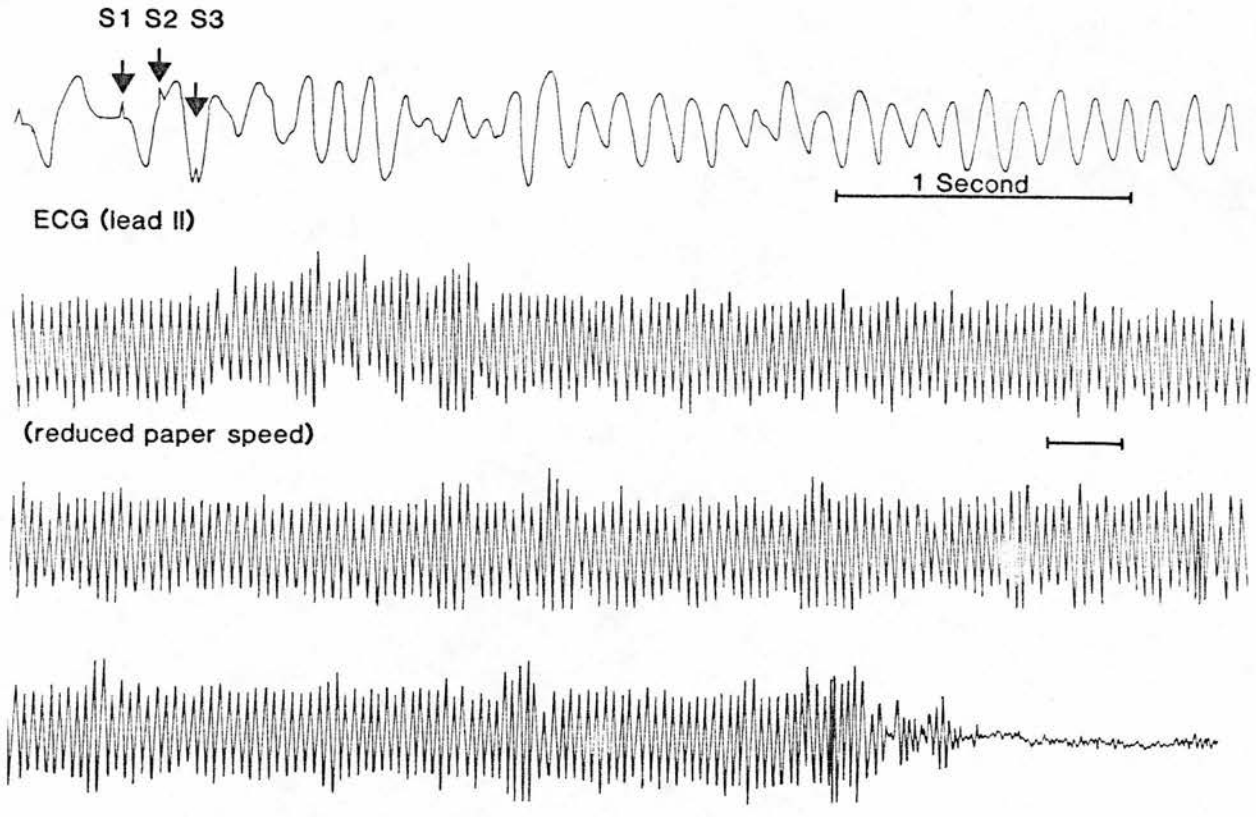


Figure 2.

Example of ventricular fibrillation following several seconds of monomorphic ventricular tachycardia in response to programmed electrical stimulation prior to administration of drug.

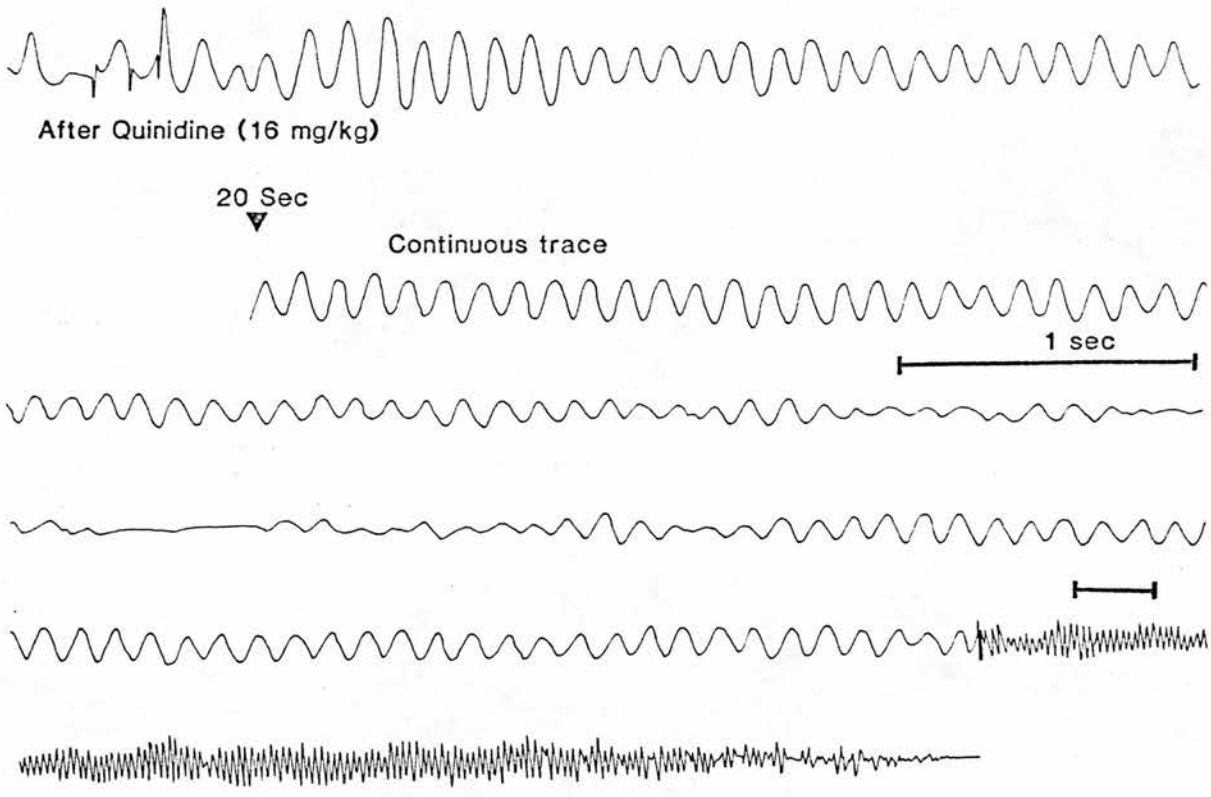
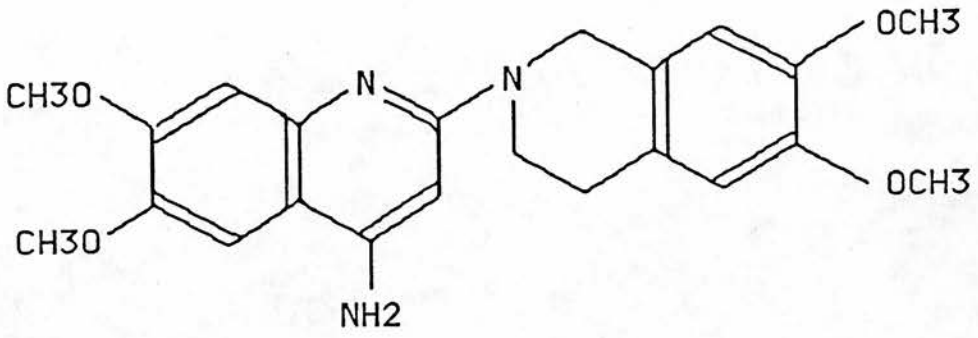


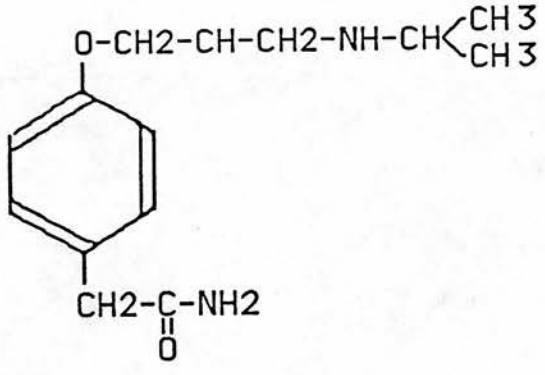
Figure 3.

Example of ventricular fibrillation following several seconds of monomorphic ventricular tachycardia in response to programmed electrical stimulation after 16 mg/kg of quinidine.

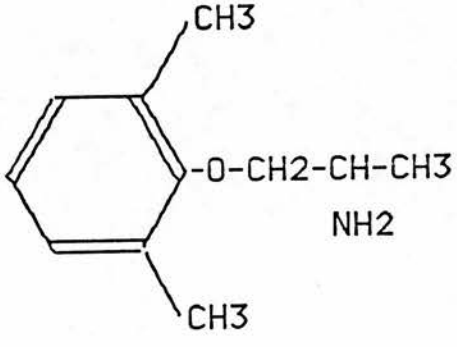
Appendix 1. Chemical structures of drugs used



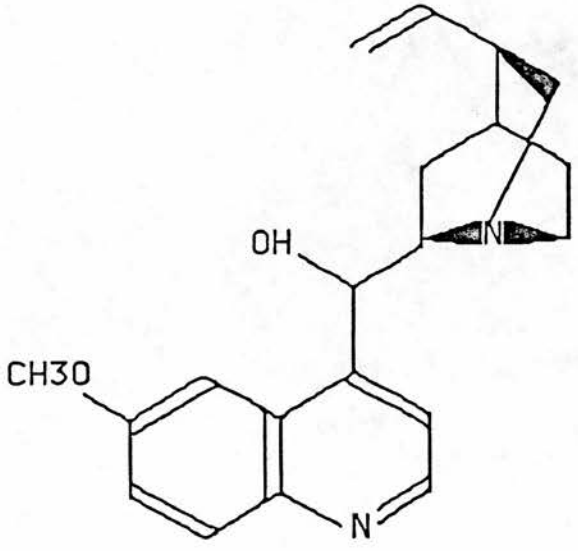
UK-52046



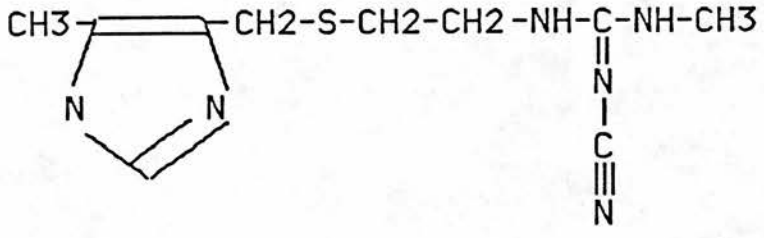
Atenolol



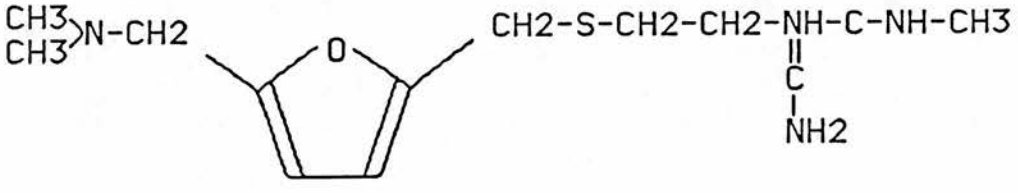
Mexiletine



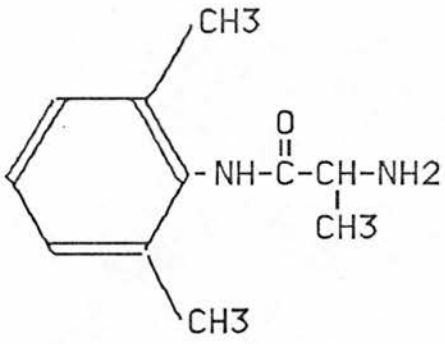
Quinidine



Cimetidine



Ranitidine



Tocainide

Appendix 2. Published papers arising as a result of this thesis

1. **Abstracts:**

Uprichard ACG, Allen JD, Harron DWG. Antiarrhythmic and electrophysiological effects of tocainide enantiomers on experimental arrhythmias produced by programmed electrical stimulation. Irish J Med Sci 1987;**156**:69-70.*

Uprichard ACG, Harron DWG. Are cimetidine and ranitidine proarrhythmic? Br J Pharmacol 1987;**91**:325P.

Uprichard ACG, Harron DWG, Shanks RG. Effects of UK-52046 and atenolol on experimental arrhythmias. PACE 1987 ;**10(4 part II)**:1021.

Uprichard ACG, Harron DWG, Shanks RG. Effects of UK-52046 on experimental reentrant arrhythmias. PACE 1987;**10(4 part II)**:1021.

Harron DWG, Grech-Belanger O, Turgeon J, Uprichard ACG. The anti-arrhythmic potency of mexiletine isomers.*FASEB J* 1988;(in press).

2. **Papers**:

Uprichard ACG, Allen JD, Harron DWG. Effects of tocainide enantiomers on experimental arrhythmias produced by programmed electrical stimulation. J Cardiovasc Pharmacol 1988; (in press).

Uprichard ACG, Harron DWG. Atenolol, but not mexiletine, protects against ventricular tachycardia and death: A study using programmed electrical stimulation in a chronic canine model. Eur Heart J 1987; (submitted).

**Winner of the Donegan first prize and medal, Royal Academy of Medicine in Ireland, Dublin 1986.*

Uprichard ACG, Harron DWG. The proarrhythmic effects of cimetidine and ranitidine: a placebo-controlled study in a model validated by the proarrhythmic effects of quinidine. Clin Cardiol 1987; (submitted).

Uprichard ACG, Harron DWG, Wilson R, Shanks RG. Effects of UK-52046 and atenolol on experimental arrhythmias. Br J Pharmacol1987; (submitted).

Uprichard ACG, Harron DWG, Wilson R, Shanks RG. Effects of UK-52046 on experimental reentrant arrhythmias. Br J Pharmacol1987; (submitted).

Bibliography

Abilgard CP. Tentamina electra in animalibus instituta. Socientatis Medicae Havniensis Collectanea 1775;**2**:157.

Agarwal JB, Akizuki S, Weintraub WS, Helfant RH. Contrasting incidence, inducibility, and transmural sites of origin of ventricular arrhythmias during acute coronary occlusion and reperfusion. Am Heart J 1984;**108(4)**:879-884.

Ahlquist RP. A study of the adrenotropic receptors. Am J Physiol 1948;**153**:586-600.

Alkondon M, Ray A, Sen P. Role of vagus in the antagonism of ouabain -induced arrhythmias in dogs by beta-adrenoceptor antagonists and and related drugs. J Pharm Pharmacol 1984; **36**:702-704.

Allen JC, Schwartz A. A possible biochemical explanation for the insensitivity of the rat to cardiac glycosides. J Pharmacol Exp Ther 1969;**168(1)**:42-46.

Allen JD, Shanks RG, Zaidi SA. The effects of lignocaine and propanolol on experimental cardiac arrhythmias. Br J Pharmacol 1971;**42**:1-12.

Allen JD, Shanks RG. Effects of tiprenolol, practolol and propanolol on experimental ventricular arrhythmias. Br J Pharmacol 1974; **51**: 179-185.

Allen JD, James RGG, McNamee BT, Shanks RG, Pantridge JF. Adrenaline-induced lignocaine-resistant dysrhythmias in experimental myocardial infarction. Am Heart J 1974;**87(1)**:21-25.

Allen JD, Brennan FJ, Wit AL. Actions of lidocaine on transmembrane potentials of subendocardial Purkinje fibers surviving in infarcted canine hearts. Circ Res 1978;**43(3)**:470-481.

Almgren O, Duker G, Reid JJ. Potency and frequency dependence of

the tocainide enantiomers in normal and high potassium. IUPHAR Meeting, Sydney, 1987;(in press).

Anderson JL, Mason JW, Winkle RA, et al.Clinical electrophysiologic effects of tocainide. Circulation 1978;**57(4)**:685-691.

Antani JA, Srinivas HV. Further evaluation of phentolamine ("Regitine") in cardiac arrhythmias. Curr Med Res Opin 1973; **1(8)**:494-500.

Au PK, Bhandari AK, Bream R, Schreck D, Siddiqi R, Rahimtoola SH. Proarrhythmic effects of antiarrhythmic drugs during programmed ventricular stimulation in patients without ventricular tachycardia. J Am Coll Cardiol 1987;**9(2)**:389-397.

Barnard RJ, Okamoto F, Buckberg GD, et al. Studies of controlled reperfusion after ischemia III. Histochemical studies: inability of triphenyltetrazolium chloride nonstaining to define tissue necrosis. J Thorac Cardiovasc Surg 1986:**92**:502-512.

Barrett AM, Cullum VA. The biological properties of the optical isomers of propanolol and their effects on cardiac arrhythmias. Br J Pharmacol 1968;**34**:43-55.

Battle WE, Naimi S, Avitall B, et al. Distinctive time course of ventricular vulnerability to fibrillation during and after release of coronary ligation. Am J Cardiol 1974;**34**:42-47.

Baum RS, Alvares H , Cobb LA. Survival after resuscitation from out-of-hospital ventricular fibrillation. Circulation 1974;**50**; 1231-1235.

Bayer R, Kalusche D, Kaufmann R, Mannhold R. Inotropic and electrophysiological actions of verapamil and D 600 in mammalian myocardium. Naunyn-Schmiedeberg's Arch Pharmacol 1975;**290**: 81-97.

Bazett HC. An analysis of the time-relations of electrocardiograms.

Heart 1920;**7**:353-370.

Beck CS, Pritchard WH, Feil HS. Ventricular fibrillation of long duration abolished by electric shock. JAMA 1947;**135(15):**985-986.

Benfey BG. Function of myocardial α-adrenoceptors. Life Sci 1982;**31**:101-112.

Benfey BG, Elfellah MS, Ogilvie RI, Varma DR. Anti-arrhythmic effects of prazosin and propanolol during coronary artery occlusion and re-perfusion in dogs and pigs. Br J Pharmacol 1984;**82**:717-725.

Bennett MA, Pentecost BL. Reversion of ventricular tachycardia by pacemaker stimulation. Br Heart J 1971;**33**:922-927.

Bergey JL, Nocella K, McCallum JD. Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. Eur J Pharmacol 1982;**81**:205-216.

Beta-Blocker Heart Attack Trial Research Group. A randomized trial of propanolol in patients with acute myocardial infarction. JAMA 1982;**247(12)**:1707-1714.

Bexton RS, Camm AJ. Drugs with a class III antiarrhythmic action. 1. Amiodarone. Pharmacol Ther 1982;**17(3)**:315-355.

Bigger JT, Weld FM, Rolnitzky LM, Ferrick KJ. Is digitalis treatment harmful in the year after myocardial infarction? Circulation 1981; **64(suppl 4)**:83(abstr).

Bigger JT, Livelli FD, Gang ES, Reiffel JA. Use of clinical electrophysiologic studies in antiarrhythmic drug development. In: Coltart DG, Jewitt DE, eds. Recent developments in cardiovascular drugs. London: Churchill-Livingstone,1982;78-93.

Bigger JT, Fleiss JL, Kleiger R, Miller JP, Rolnitzky LM, Multicenter Postinfarction Research Group. The relationships among ventricular

arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. Circulation 1984;**69(2)**:250-280.

Black JW, Duncan WAM, Durant GJ, Ganellin CR, Parsons EM. Definition and antagonism of histamine H2-receptors. Nature 1972;**236**: 385-390.

Block AJ. Prevention of chloroform-induced ventricular tachycardia in mice as an index of antiarrhythmic activity. Life Sci 1981;**28:** 2623-2629.

Boineau J, Cox J. Slow ventricular activation in acute myocardial infarction. Circulation 1973;**47**:702-713.

Bournerias F, Ganeval D, Danan G. Trouble du rythme cardiaque mortel au cours d'un traitement par la cimetidine. Nouvelle Presse Med 1978;**7**:2069.

Brachmann J, Senges J, Spath J, et al. Effect of tocainide on normal and damaged ventricle in conscious dogs during the late postinfarction phase. Eur Heart J 1984;**5(suppl 8)**:116(abstr).

Braunwald E. Mechanism of action of calcium-channel-blocking agents. New Engl J Med 1982;**307(26)**:1618-1627.

Breithardt G, Seipel L, Abendroth RR. Comparison of the antiarrhythmic efficacy of disopyramide and mexiletine against stimulus-induced ventricular tachycardia. J Cardiovasc Pharmacol 1981;**3(5)**:1026-1037.

Broadley KJ. The Langendorff heart preparation - reappraisal of its role as a research and teaching model for coronary vasoactive drugs. J Pharmacol Methods 1979;**2**:143-156.

Brooks WW, Verrier RL, Lown B. Protective effect of verapamil on vulnerability to ventricular fibrillation during myocardial ischaemia and reperfusion. Cardiovasc Res 1980;**14(5)**:295-302.

Buxton AE, Josephson ME. Role of electrophysiologic studies in identifying arrhythmogenic properties of antiarrhythmic drugs. Circulation 1986;**73(suppl II)**:II.67-II.71.

Byrnes EW, McMaster PD, Smith ER, et al. New antiarrhythmic agents.1.Primary α-amino anilides. J Med Chem 1979;**22(10)**: 1171-1176.

Camarri E, Chirone E, Fanteria G, Zocchi M. Ranitidine induced bradycardia. Lancet 1982;**ii**:160.

Cameron JS, Dersham GH, Han J. Effects of epinephrine on the electrophysiologic properties of Purkinje fibers surviving myocardial infarction. Am Heart J 1982;**104(3)**:551-560.

Cameron JS, Gaide MS, Altman CB, Cuevas J, Myerburg RJ, Bassett AL. Cellular electrophysiologic effects of histamine following myocardial infarction in the guinea pig. Physiologist 1983a;**26**: A-100(abstr).

Cameron JS, Gaide MS, Altman CB, Myerburg AL, Bassett AL. Cimetidine blocks the adverse electrophysiologic effects of histamine in vitro after myocardial infarction in guinea pigs. Circulation 1983b;**68**:III-221(abstr).

Campbell RWF. Recent development in antiarrhythmic therapy-mexiletine, disopyramide and flecainide. In: Coltart J, Jewitt OE, eds. Recent developments in cardiovascular drugs. London: Churchill-Livingstone, 1982;94-101.

Carlson HA, Ippiliti AF. Cimetidine, an H2 antihistamine, stimulates prolactin secretion in man. J Clin Endocr & Metab 1977;**45(2)**: 367-370.

Carmeliet E, Vanhouette F, Nerbeke N. Enantioselective electro-physiological properties of tocainide. Br J Pharmacol 1986;**89**: 700P(abstr).

Chardack WM, Gage AA, Federico AJ, Cusick JK, Matsumoto PJH, Lanphier EH. Reduction by hyperbaric oxygenation of the mortality from ventricular fibrillation following coronary artery ligation. Circ Res 1964;**15**:497-502.

Cheung W, Maroko PR, Hastie R, Day C. Bepridil, a new calcium antagonist, reduces sudden death in the atherosclerotic quail model. Circulation 1983;**68(4)**:III-271(abstr).

Chung EKY. Parasystole. Progr Cardiovasc Dis 1968;**11(1)**:64-81.

Cobbe SM, Hoffmann E, Ritzenhoff A, Brachmann J, Kubler W, Senges J. Day-to-day variations in inducibility of ventricular tachyarrhythmias during the late postmyocardial infarction phase in conscious dogs. Circulation1985;**72(1)**:200-204.

Cohen J, Weetman AP, Dargie HJ, Krikler DM. Life-threatening arrhythmias and intravenous cimetidine. Br Med J 1979;**2**:768.

Coker SJ. Preliminary studies on ischaemia and reperfusion-induced dysrhythmias in anaesthetised rabbits. Br J Pharmacol 1987;**90**:21P(abstr).

Coltard DJ, Berndt TB, Kernoff R, Harrison DC. Antiarrhythmic and circulatory effects of Astra W36095. Am J Cardiol 1974;**34**:35-41.

Constantin L, Martins JB. Autonomic control of ventricular tachy-cardia: direct effects of beta-adrenergic blockade in 24 hour old canine myocardial infarction. J Am Coll Cardiol 1987;**9(2)**:366-373.

Corr PB, Gillis RA. Autonomic neural influences on the dysrhythmias resulting from myocardial infarction. Circ Res1978;**43(1)**:1-9.

Corr PB, Shayman JA, Kramer JB, Kipnis RJ. Increased α-adrenergic receptors in ischemic cat myocardium. J Clin Invest 1981;**67**:1232-1236.

Corr PB, Sharma AD. Alpha-adrenergic-mediated effects on

myocardial calcium. In: Opie LH, ed. Calcium antagonists and cardiovascular disease. New York: Raven Press,1984;193-204.

Couch OA. Cardiac aneurysm with ventricular tachycardia and subsequent excision of aneurysm: case report. Circulation 1959;**20**:251-253.

Craft TM. Torsade de pointes after astemizole overdose. Br Med J 1986;**292**:660.

Cranefield PF. Action potentials, afterpotentials, and arrhythmias. Circ Res 1977;**41(4)**:415-423.

Cranefield PF. The slow inward current and the generation of cardiac arrhythmias. In: Richardson RG, ed. Ca^{++} Antagonism: The proceedings of a one-day symposium. Queensborough: Abbott Laboratories,1980;15-23.

Curtis MJ, MacLeod BA, Walker MJA. Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. Br J Pharmacol 1984;**83**: 373-385.

Dadkar NK, Bhattacharya BK. A rapid screening procedure for antiarrhythmic activity in the mouse. Arch Int Pharmacodyn 1974;**212**: 297-301.

Damato AM, Lau S, Bobb GA. Digitalis-induced bundle branch ventricular tachycardia studied by electrode catheter recordings of the specialized conducting tissue of the dog. Circ Res 1971;**28**:16-22.

Davey MJ. Cardiovascular pharmacology of prazosin - a selective alpha 1-adrenergic receptor blocking agent. In: Vanhoutte PM, Leusen I, eds. Vasodilatation. New York: Raven Press,1981;499-510.

Davis CL. Development of the human heart from its first appearance to the state found in embryos of 20 paired somnites. Contrib Embryol 1927;**19**:245-284.

Davis J, Glassman R, Wit AL. Method for evaluating the effects of antiarrhythmic drugs on ventricular tachycardias with different electrophysiologic characteristics and different mechanisms in the infarcted canine heart. Am J Cardiol 1982;**49**:1176-1184.

Davis LD, Helmer PR, Ballantyne F. Production of slow responses in canine cardiac Purkinje fibers exposed to reduced pH. J Moll Cell Cardiol 1976;**8**:61-76.

Davis LD, Temte JV, Murphy QR. Epinephrine-cyclopropane effects on Purkinje fibers. Anesthesiology 1969;**30(4)**:369-377.

DeBoer LWV, Nosta JJ, Kloner RA, Braunwald E. Studies of amiodarone during experimental myocardial infarction: beneficial effects on hemodynamics and infarct size. Circulation 1982:**65(3)**: 508-512.

DiBianco R, Fletcher RD, Cohen AI, et al. Treatment of frequent ventricular arrhythmia with encainide: Assessment using serial ambulatory electrocardiograms, intracardiac electrophysiologic studies, treadmill exercise tests, and radionuclide cineangiographic studies. Circulation 1982;**65(6)**:1134-1147.

DiCarlo L, Lynch JJ, Montgomery D, Lucchesi BR. Effect of flecainide acetate on induced ventricular tachycardia and ventricular fibrillation in dogs with recent myocardial infarction. Circulation 1985;**72**: III-225(abstr).

DiMarco JP, Garan H, Ruskin J. Mexiletine for refractory ventricular arrhythmias:Results using serial electrophysiologic testing. Am J Cardiol 1981;**47**:131-138.

DiMicco JA, Prestel T, Pearle DL, Gillis RA. Mechanism of cardiovascular changes produced in cats by activation of the central nervous system with picrotoxin. Circ Res 1977;**41(4)**:446-451.

Dobmeyer DJ, Stine RA, Leier CV, Schaal SF. Arrhythmogenic

effects of caffeine in man as determined by programmed electrictrical stimulation. Clin Res 1982;**30(2)**:183(abstr).

Doyle JT, Kannel WB, McNamara PM, Quickenton P, Gordon T. Factors related to sudden death from coronary disease: combined Albany-Framingham studies. Am J Cardiol 1976;**37**:1073-1078.

Dreifus LS, Naito M, Michelson EL. What animal models should be used to define antiarrhythmic efficacy? Acute dog models. In: Morganroth J, Moore EN, Dreifus LS, Michelson EL, eds.The evaluation of new antiarrhythmic drugs. The Hague: Martinus Nijhoff,1981;17-32.

Dresel PE, MacCannell KL, Nickerson M. Cardiac arrhythmias induced by minimal doses of epinephrine in cyclopropane-anesthetized dogs. Circ Res 1960;**8**:948-955.

Dresel PE, Sutter MC. Factors modifying cyclopropane-epinephrine cardiac arrhythmias. Circ Res 1961;**9**:1284-1290.

Duff HJ, Roden DM, Dawson AK, Oates JA, Smith RF, Woosley RL. Comparison of the effects of placebo and encainide on programmed electrical stimulation and ventricular arrhythmia frequency.Am J Cardiol 1982;**50**:305-312.

Dukes ID, Vaughan Williams EM. Electrophysiological effects of alpha-adrenoceptor antagonists in rabbit sino-atrial node, Purkinje cells and papillary muscle. Br J Pharmacol 1984;**83**:419-426.

Dutta S, Marks BH. Distribution of ouabain and digoxin in the rat and guinea-pig. Life Sci 1966;**5**:915-920.

Easley A, Higgins S, Thieme L, Lindenfeld J, Reiter MHJ. Tocainide in preventing inducible ventricular tachycardia in unselected patients. Clin Res 1985;**33(1)**:6A(abstr).

Ebert PA, Vanderbeek RB, Allgood RJ, Sabiston DC. Effect of chronic cardiac denervation on arrhythmias after coronary artery ligation. Cardiovasc Res 1970;**4**:141-147 .

Echt DS, Griffin JC, Ford AJ, Knutti JW, Feldman RC, Mason JW. Nature of inducible ventricular tachyarrhythmias in a canine chronic myocardial infarction model. Am J Cardiol 1983;**52**:1127-1132.

Echt DS, Mason JW. Management of serious cardiac arrhythmias with drugs. Cardiovasc Clin 1984;**14(3)**:191-209.

Edvardsson N, Olsson SB. Effects of acute and chronic beta-receptor blockade on ventricular repolarisation in man. Br Heart J 1981;**45**: 628-636.

Ehsani A, Ewy GA, Sobel BE. Effects of electrical countershock on serum creatine phosphokinase (CPK) isoenzyme activity. Am J Cardiol 1976;**37**:12-18.

Eisenberg MS, Bergner L, Hallstrom AP, Cummins RO. Sudden cardiac death. Sci Am 1986;**254(5)**:25-31.

El-Sherif N, Scherlag BJ, Lazzara R, Hope RR. Re-entrant ventricular arrhythmias in the late myocardial infarction period 1. Conduction characteristics in the infarction zone. Circulation 1977a;**55(5)**: 686-702.

El Sherif N, Hope RR, Scherlag BJ, Lazzara RR. Re-entrant ventricular arrhythmias in the late myocardial infarction period 2. Patterns of initiation and termination of re-entry. Circulation 1977b;**55(5)**: 702-719.

El-Sherif N. Re-entrant ventricular arrhythmias in the late myocardial infarction period 6. Effect of the autonomic system. Circulation 1978; **58(1)**:103-110.

El-Sherif N, Lazzara R. Re-entrant ventricular arrhythmias in the late myocardial infarction period 7. Effect of verapamil and D-600 and the role of the "slow channel".Circulation 1979;**60(3)**:605-615.

Engel TR, Luck JC. Histamine 2 receptor antagonism by cimetidine

and sinus node function. New Engl J Med 1979;**301(11)**:591-592.

Erichsen JE. On the influence of the coronary circulation on the action of the heart. Lond Med Gaz 1842;**2**:561-564.

Euler DE, Zeman TW, Wallock ME, Scanlon PJ. Deleterious effects of bretylium on haemodynamic recovery from ventricular fibrillation. J Am Coll Cardiol 1986;**7**:250A(abstr).

Fenoglio JJ, Silva FG, Friedman PL, Wit AL. Survival of subendocardial Purkinje fibers following extensive myocardial infarction in humans. Hum Pathol 1976;**7:**547-563.

Fenster PE, Kern KB. Mexiletine in refractory ventricular arrhythmias. Clin Pharmacol Ther 1983;**34(6)**:777-784.

Ferrier GR, Dresel PE. Relationship of the functional refractory period to conduction in the atrioventricular node. Circ Res 1974;**35**:204-214.

Ferris L, King BG, Spence PW, Williams HB. Effect of electrical shock on the heart. Electl Engng 1936; **55**:498-515.

Fishbein MC, Meerbaum S, Rit J, et al. Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. Am Heart J 1981;**101(5)**: 593-600.

Flameng W, Vanhaecke J, Vandeplassche G. Studies on experimental myocardial infarction:dogs or baboons? Cardiovasc Res 1986;**20**: 241-247.

Fondacaro JD, Han J, Yoon MS. Effects of verapamil on ventricular rhythm during acute coronary occlusion.Am Heart J 1978;96(1):81-86.

Fontaine G, Guiraudon G, Frank R, Cabrol C, Grosgogeat Y. The surgical

management of ventricular tachycardia. Herz 1979;**4**:276-284.

Friedman L, Yusuf S. Does therapy directed by programmed electrical stimulation provide a satisfactory clinical response? Circulation 1986;**73(suppl II)**:II.59-II.65.

Friedman M, Mainwaring JH, Rosenman RH, Donlon G, Ortega P, Grube SM. Instantaneous and sudden deaths: Clinical and pathological differentiation in coronary artery disease. JAMA 1973;**225(11)**: 1319-1328.

Friedman PL, Stewart JR, Wit AL. Spontaneous and induced cardiac arrhythmias in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. Circ Res 1973;**33**:612-626.

Furberg CD. Effects of antiarrhythmic drugs on mortality after myocardial infarction. Am J Cardiol 1983;**52**:32C-36C.

Gal J, French TA, Zysset T, Haroldsen PE. Disposition of (R,S) -tocainide. Some stereoselective aspects. Drug Metab Disp 1982; **10(4)**:399-404.

Gallagher JJ. Surgical treatment of arrhythmias: current status and future directions. Am J Cardiol 1978;**41**:1035-1044.

Gallagher JJ. Mechanisms of arrhythmis and conduction abnormalities. In: Hurst JW, ed. The heart. New York: McGraw-Hill,1982;489-519.

Gamble OW, Cohn K. Effect of propanolol, procainamide and lidocaine on ventricular automaticity and reentry in experimental myocardial infarction. Circulation 1972;**46**:498-506.

Gang ES, Bigger JT, Livelli FD. A model of chronic ischemic arrhythmias: the relation between electrically inducible ventricular tachycardia, ventricular fibrillation threshold and myocardial infarct size. Am J Cardiol 1982;**50**:469-477.

Gang ES, Bigger JT, Uhl EW. Effects of timolol and propanolol on inducible sustained ventricular tachyarrhythmias in dogs with subacute myocardial infarction. Am J Cardiol 1984;**53**:275-281.

Garan H, Fallon JT, Ruskin JN. Sustained ventricular tachycardia in recent canine myocardial infarction. Circulation 1980;**62(5)** 980-987.

Garan H, Fallon JT, Ruskin JN. Nonsustained polymorphic ventricular tachycardia induced by electrical stimulation in 3 week old canine myocardial infarction. Am J Cardiol 1981;**48**:280-286.

Gettes LS. On the classification of antiarrhythmic drugs. Mod Concepts Cardiovasc Dis 1979;**48(3)**:13-18.

Gettes LS. Effect of ischemia on cardiac electrophysiology. In: Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE,eds. The heart and cardiovascular system. New York: Raven Press,1986;1317-1341.

Gibson JK, Lucchesi BR. electrophysiologic actions of UM-272 (Pranolium) on re-entrant ventricular arrhythmias in post-infarction canine myocardium. J Pharmacol Exp Ther 1980;**214(2)**:347-353.

Gillis AM, Clusin WT. The role of beta-adrenergic receptor antagonists in the prevention of sudden cardiac death. Compr Ther 1984;**10(9)**: 60-67.

Ginks WR, Sybers HD, Maroko PR, Covell JW, Sobel BE, Ross J. Coronary artery reperfusion 2. Reduction of myocardial infarct size at 1 week after the coronary occlusion. J Clin Invest 1972;**51**:2717-2723.

Glassman RD, Davis JC, Wit AL. Effects of antiarrhythmic drugs on sustained ventricular tachycardia induced by a premature stimulus in dogs after coronary artery occlusion and reperfusion. Fed Proc 1978; **37**:730(abstr).

Gloor HO, Urthaler F, James TN. Acute effects of amiodarone upon the

canine sinus node and atrioventricular junctional region. J Clin Invest 1983;**71**:1457-1466.

Goldstein RE, Karsh RB, Smith ER, et al. Influence of atropine and of vagally mediated bradycardia on the occurence of ventricular arrhythmias following acute coronary occlusion in closed-chest dogs. Circulation 1973;**47**:1180-1190.

Goldstein RE, Tibbits PA, Oetgen WJ. Proarrhythmic effects of antiarrhythmic drugs. Ann NY Acad Sci 1984;**427**:94-100.

Goldstein S. The necessity of a uniform definition of sudden coronary death: Witnessed death within 1 hour of the onset of acute symptoms. Am Heart J 1982;**103**:156-159.

Gordon T, Kannel WB. Premature mortality from coronary heart disease. The Framingham study. JAMA 1971;**215(10)**:1617-1625.

Goshima K, Wakabayashi S. Inhibition of ouabain-induced increase in Na content of cultured myocardial cells by quinidine and procainamide. J Pharmacol Exp Ther 1983;**224(1)**:239-246.

Gould L, Reddy CVR, Weinstein T, Gomprecht RF. Antiarrhythmic prophylaxis with phentolamine in acute myocardial infarction. J Clin Pharmacol 1975;**15**:191-197.

Gould L, Reddy CVR, Singh BK, Zen B. Electrophysiologic properties of cimetidine in man. PACE 1981;**4**:3-7.

Govier WC. A positive inotropic effect of phenylephrine mediated through alpha adrenergic receptors. Life Sci 1967;**6**:1361-1365.

Govier WC. Myocardial alpha adrenergic receptors and their role in the production of a positive inotropic effect by sympathomimetic agents. J Pharmacol Exp Ther 1968;**159(1)**:82-90.

Gradman AH, Bell PA, De Busk RF. Sudden death during ambulatory monitoring. Clinical and electrocardiographic correlations. Report

of a case. Circulation 1977;**55**:210-211.

Grech-Belanger O, Turgeon J, Gilbert M. Stereoselective disposition of mexiletine in man. Br J Clin Pharmacol 1986;**21**:481-487.

Griffin JC, Mason JW, Ross DL, Calfee RV. The treatment of ventricular tachycardia using an automatic tachycardia terminating pacemaker. PACE 1981;**4**:582-588.

Hamer AW, Karagueuzian HS, Sugi K, Zaher CA, Mandel WJ, Peter T. Factors related to the induction of ventricular fibrillation in the normal canine heart by programmed electrical stimulation. J Am Coll Cardiol 1984;**3(3)**:751-759.

Harris AS. Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. Circulation 1950;**1**: 1318-1329.

Harrison DC. Symposium on perspectives on the treatment of ventricular arrhythmias. Am J Cardiol 1983;**52(6)**:1C-2C.

Hart G. Physiology of cardiac conduction. Br J Hosp Med 1985;**35**:128-137.

Hashimoto K, Hashimoto K. The mechanism of sensitization of the ventricle to epinephrine by halothane. Am Heart J1972;**83(5)**: 652-658.

Hashimoto K, Satoh H, Shibuya T, Imai S. Canin-effective plasma concentrations of antiarrhythmic drugs on the two-stage coronary ligation arrhythmia. J Pharmacol Exp Ther 1982;**222(3)**:801-810.

Hearse DJ. Models and problems in the study of myocardial ischemia and tissue protection. Eur Heart J 1983;**4**:43-48.

Heissenbuttel RH, Bigger JT. The effect of oral quinidine on intraventricular conduction in man: correlation of plasma quinidine with changes in QRS duration. Am Heart J 1970;**80(4)**:453-462.

Helmers C, Lundman T, Maasing R, Wester PO. Mortality pattern among initial survivors of acute myocardial infarction using a life-table technique. Acta Med Scand 1976;**200**:469-473.

Hermansen K. Evidence for adrenergic mediation of ouabain induced arrhythmias in the guinea pig. Acta Pharmacol Toxicol 1970;**28**: 57-65.

Heusch G, Deussen A, Schipke J, Thamer V. α1- and α2-adrenoceptor-
mediated vasoconstriction of large and small canine coronary arteries in vivo. J Cardiovasc Pharmacol 1984;**6**:961-968.

Hinkle LE, Argyros DC, Hayes JC, Robinson T, Alonso DR. Pathogenesis of an unexpected sudden death: Role of early cycle ventricular premature contractions. Am J Cardiol 1977;**39**:873-879.

Hinkle LE. The immediate antecedents of sudden death. Acta Med Scand (suppl) 1981;**651**:207-217.

Hoffman BF, Cranefield PF. Electrophysiology of the heart. New York: Futura,1960.

Hoffman BF, Singer DH. Appraisal of the effects of catecholamines on cardiac electrical activity. Ann NY Acad Sci 1967;**139**:914-939.

Hoffman BF, Bigger JT. Antiarrhythmic drugs. In: DiPalma JR, ed. Drill's pharmacology in medicine. New York: McGraw-Hill,1971; 824-852.

Hoffman BF, Rosen MR. Cellular mechanisms for cardiac arrhythmias. Circ Res 1981;**49(1)**:1-15.

Hoffman BF, Dangman KH. The role of antiarrhythmic drugs in sudden cardiac death. J Am Coll Cardiol 1986;**8(1)**:104A-109A.

Holtz J, Saeed M, Sommer O, Bassenge E. Norepinephrine constricts

the canine coronary bed via postsynaptic alpha-2 receptors. Eur J Pharmacol 1982;**82**:199-202.

Hope RR, Williams DO, El-Sherif N, Lazzara R, Scherlag BJ. The efficacy of antiarrhythmic agents during acute myocardial ischemia and the role of heart rate. Circulation 1974;**50**:507-514.

Horowitz LN, Spear JF, Moore EN. Subendocardial origin of ventricular arrhythmias in 24-hour-old experimental myocardial infarction. Circulation 1976;**53(1)**:56-63.

Horowitz LN, Josephson ME, Farshidi A, Spielman SR, Michelson EL, Greenspan AM. Recurrent sustained ventricular tachycardia 3. Role of the electrophysiologic study in selection of antiarrhythmic regimens. Circulation 1978;**58(6)**:986-997.

Horowitz LN, Harken AH, Kastor JA, Josephson ME. Ventricular resection guided by epicardial and endocardial mapping for treatment of recurrent ventricular tachycardia. New Engl J Med 1980;**302(11)**: 589-593.

Howe R. Structure activity relationships of some β-adrenergic blocking agents. Biochem Pharmacol 1966;**12(suppl 85)**: 85-86(abstr).

Howe R, Shanks RG. Optical isomers of propanolol. Nature (London) 1966;**210**:1336-1338.

Howitt G, Husaini M, Rowlands DJ, Logan WFWE, Shanks RG, Evans MG. The effect of the dextro isomer of propanolol on sinus rate and cardiac arrhythmias. Am Heart J 1968;**76(6)**:736-745.

Hurst JW, King JB, Walter PF, Friesinger GC, Edwards JE. Atherosclerotic coronary heart disease: angina pectoris, myocardial infarction and other manifestations of myocardial ischemia. In: Hurst JW, ed. The heart. New York: McGraw-Hill,1982;1009-1149.

IMPACT Research Group. International mexiletine and placebo

antiarrhythmic coronary trial: 1.Report on arrhythmia and other findings. J Am Coll Cardiol 1984;**4(6)**:1148-1163.

Inque F, MacLeod BA, Walker MJA. Intracellular potential changes following coronary occlusion in isolated perfused rat hearts Can J Physiol Pharmacol 1984;**62**:658-664.

ISIS-1 (First International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous atenolol among 16,027 cases of suspected acute myocardial infarction. Lancet 1986;**ii**:57-66.

Jennings RB, Reimer KA. Lethal myocardial ischemic injury. Am J Pathol 1981;**102**:241-255.

Jenzer HR, Hagemeijer F. Quinidine syncope: torsade de pointes with low quinidine plasma concentrations. Eur J Cardiol 1976;**4**:447-451.

Josephson ME, Horowitz LN, Farshidi A, Kastor JA. Recurrent sustained ventricular tachycardia. 1. Mechanisms. Circulation 1978a;**57(3)**: 431-440.

Josephson ME, Horowitz LN, Farshidi A. Continuous local electrical activity: A mechanism of recurrent ventricular tachycardia. Circulation 1978b;**57(4)**:659-665.

Juhasj-Nagy A, Aviado DM. Increased role of alpha-adrenoceptors in ischemic myocardial zones. Physiologist 1976;**19**:245(abstr).

Julian DG. Cardiology. London: Bailliere Tindall,1979.

Julian DG. Prevention of sudden death. In: Harrison DC, ed. Cardiac arrhythmias. Boston: GK Hall,1981;111-117.

Julian DG. Prospect for prevention of arrhythmias complicating the acute coronary attack. In: Geddes JS, ed. The management of the acute coronary attack. London: Academic Press,1986;51-61.

Kabell G, Brachmann J, Scherlag BJ, Harrison L, Lazzara R. Mechanism

of ventricular arrhythmias in multivessel coronary disease: the effects of collateral zone ischemia. Am Heart J 1984;**108**:447-454.

Kane KA, Winslow E. Antidysrhythmic and electrophysiological effects of a new antianginal agent, Bepridil. J Cardiovasc Pharmacol 1980;**2**:193-203.

Kaplinsky E, Ogawa S, Balke CW, Dreifus LS. Two periods of early ventricular arrhythmia in the canine acute myocardial infarction model. Circulation 1979;**60(2)**:397-403.

Karagueuzian HS, Fenoglio JJ, Weiss MB, Wit AL. Protracted ventricular tachycardia induced by premature stimulation of the canine heart after coronary artery occlusion and reperfusion. Circ Res 1979;**44(6)**:833-846.

Karagueuzian HS, Fugimoto T, Katoh T, Peter T, McCullen A, Mandel WJ. Suppression of ventricular arrhythmias by propafenone, a new antiarrhythmic agent, during acute myocardial infarction in the conscious dog: a comparative study with lidocaine. Circulation 1982; **66(6)**:1190-1198.

Karsh FE, Becker LA. Bradycardia associated with cimetidine. J Fam Pract1980;**11(2)**:319-320.

Kasanuki H, Ohnishi S, Hirosawa K. Availability of electrophysiologic approach to the selection and assessment of antiarrhythmic drugs for recurrent ventricular tachycardia. Jpn Circ J 1983;**47(1)**:105-123.

Kempf FC, Josephson ME. Cardiac arrest recorded on ambulatory monitoring. Am J Cardiol 1984;**53**:1577-1582.

Khan MM, Logan KR, McComb JM, Adgey AAJ. Management of recurrent ventricular arrhythmias associated with QT prolongation. Am J Cardiol 1981;**47**:1301-1308.

Kim SG, Seidon SW, Felder SD, Waspe LE, Fisher JD. Is programmed

stimulation of value in predicting the long-term success of antiarrhythmic therapy for ventricular tachycardias? New Engl J Med 1986;**315(6)**:356-362.

King AR. Cardiac arrest and cimetidine. Med J Aust 1981;**1**:139-140.

Kjekshus J. Comments - beta blockers: heart rate reduction a mechanism of benefit. Eur Heart J 1985;**6(suppl A)**:29-30.

Kniffen FJ, Lomas TE, Counsell RE, Lucchesi BR. The antiarrhythmic and antifibrillatory actions of bretylium and its O-iodobenzyl tetramethylammonium analog, UM-360. J Pharmacol Exp Ther 1975; **192**:120-128.

Konzett H, Rothlin E. Effect of cardioactive glycosides on a sympathetic ganglion. Arch Int Pharmacodyn 1952;**89(3)**:343-352.

Kralios FA, Martin L, Burgess MJ, Millar K. Local ventricular repolarization changes due to sympathetic nerve-branch stimulation. Am J Physiol 1975;**228(5)**:1621-1626.

Krikler DM, Spurrell RAJ. Verapamil in the treatment of paroxysmal supraventricular tachycardia. Postgrad Med J 1974;**50**:447-453.

Kuller L. Sudden and unexpected non-traumatic deaths in adults: a review of epidemiological and clinical studies. J Chron Dis 1966;**19**: 1165-1192.

Kuller L, Cooper M, Perper J. Epidemiology of sudden death. Arch Int Med 1972;**129**:714-719.

Langer SZ. Presynaptic receptors and their role in the regulation of transmitter release. Sixth Gaddum Memorial Lecture. Br J Pharmacol 1977;**60**:481-497.

Lazzara R, Scherlag BJ. Treatment of arrhythmias by blocking slow current. Ann Intern Med 1980;**93(6)**:919-921.

Leimdorfer A. Prevention of cardiac arrhythmias by regitine. Arch Int Pharmacodyn 1953;**94(1)**:119-126.

Leslie GB, Walker TF. A toxicological profile of cimetidine. In: Second international symposium on H2 receptor antagonist. Amsterdam: Excerpta Medica, 1977;24-31.

Levi R. Effects of exogenous and immunologically released histamine on the isolated heart: A qualitative comparison. J Pharmacol Exp Ther 1972;**182(2)**:227-238.

Levi R, Allan G, Zavecz JH. Cardiac histamine receptors. Fed Proc 1976;**35(3)**:1942-1947.

Levi R, Malm JR, Bowman FO, Rosen MR. The arrhythmogenic actions of histamine on human atrial fibers. Circ Res 1981;**49(2)**:545-550.

Liberthson RR, Nagel EL, Hirschman JC, Nussenfeld SR. Prehospital ventricular defibrillation: prognosis ans follow-up course. New Engl J Med 1974;**291**:317-321.

Lie JT, Pairolero PC, Holley KE, Titus JL. Macroscopic enzyme-mapping verification of large, homogeneous, experimental myocardial infarcts of predictable size and location in dogs. J Thorac Cardiovasc Surg 1975;**69**;599-605.

Ligumsky M, Shochina M, Rachmilewitz D. Cimetidine and arrhythmia suppression. Ann Intern Med 1978;**89(6)**:1008-1009.

Lineberger AS, Sprague DH, Battaglini JW. Sinus arrest associated with cimetidine. Anesth Analg 1985;**64(5)**:554-556.

Lown B, Neumann J, Amarasingham R, Berkovits BV. Comparison of alternating current with direct current electroshock across the closed chest. Am J Cardiol 1962;**10**:223-233.

Lown B, Verrier RL. Neural activity and ventricular fibrillation. New Engl J Med 1976;**294(21)**:1165-1170.

Lown B, Graboys TB. Sudden death: an ancient problem newly perceived. Cardiovasc Med 1977;**2(3)**:219-233.

Lown B, Graboys TB, Podrid PJ, Lampert S, Blatt CM. Historic perspectives- electrical stimulation of the heart. Circulation 1986;**72(suppl II)**:II.3-II.10.

Lubbe WF, Daries PS, Opie LH. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc Res 1978;**12**:212-220.

Lucchesi BR. The effects of pronethalol and its dextro isomer upon experimental cardiac arrhythmias. J Pharmacol Exp Ther 1965; **148(1)**:94-99.

Lucchesi BR. Sudden cardiac death: pharmacological interventions for the prevention of ventricular fibrillation. TIPS 1984;**5(4)**:145-148.

Lynch JJ, Wilber DJ, Montgomery DG, Hsieh TM, Patterson E, Lucchesi BR. Antiarrhythmic and antifibrillatory actions of the levo- and dextrorotatory isomers of sotalol. J Cardiovasc Pharmacol 1984;**6**: 1132-1141.

Lynch JJ, Montgonery DG, Lucchesi BR. Facilitation of lethal ventricular arrhythmias by therapeutic digoxin in conscious post infarction dogs. Am Heart J 1986;**111(5)**:883-890.

Lynch JJ, Nelson SD, MacEwen SA, Driscoll EM, Lucchesi BR. Antifibrillatory efficacy of concomitant beta adrenergic receptor blockade with dilevalol, the R,R-isomer of labetalol, and muscarinic receptor blockade with methylscopolamine. J Pharmacol Exp Ther 1987;**241(2)**:741-749.

Lynch JJ, Lucchesi BR. How are animal models best used for the study of antiarrhythmic drugs? In: Hearse DJ, Manning AS, Janse MJ, eds. Life-threatening arrhythmias during ischemia and infarction. New York: Raven Press,1987;169-196.

MacMahon B, Bakshi M, Walsh MJ. Cardiac arrhythmias after intravenous cimetidine. New Engl J Med 1981;**305(14)**:832-833.

McAllister RE, Nobel D, Tsien RW. Reconstruction of the electrical activity of cardiac Purkinje fibres. J Physiol1975;**251**:1-59.

McCallister BD, McGoon DC, Connolly DC. Paroxysmal ventricular tachycardia and fibrillation without complete heart block. Am J Cardiol 1966;**18**:898-903.

McEachern CG, Manning GW, Hall GE. Sudden occlusion of coronary arteries following removal of cardio-sensory pathways. Arch Int Med 1940;**65**:661-670.

McWilliam JA. Cardiac failure and sudden death. Br Med J 1889;**1**:6-8.

Madsen JK. Ischaemic heart disease and prodromes of sudden cardiac death. Br Heart J 1985;**54**:27-32.

Maling HM, Moran NC. Ventricular arrhythmias induced by sympathomimetic amines in unanesthetized dogs following coronary artery occlusion. Circ Res 1957;**5**:409-413.

Maling HM, Cohn VH, Highman B. The effects of coronary occlusion in dogs treated with reserpine and in dogs treated with phenoxybenzamine. J Pharmacol Exp Ther 1959;**127**:229-235

Manning GW, McEachern CG, Hall GE. Reflex coronary artery spasm following sudden occlusion of other coronary branches. Arch Int Med 1939;**64**:661-674.

Marchlinsky FE, Buxton AE, Waxman HL, Josephson ME. Identifying patients at risk of sudden death after myocardial infarction: value of the response to programmed stimulation, degree of ventricular ectopic activity and severity of left ventricular dysfunction. Am J Cardiol 1983;**52**:1190-1196.

Maroko PR, Libby P, Ginks WR, et al. Coronary artery reperfusion 1. Early effects on local myocardial function and the extent of myocardial necrosis. J Clin Invest 1972;**51**:2710-2716.

Martins JB. Autonomic control of ventricular tachycardia: sympathetic neural influence on spontaneous tachycardia 24 hours after coronary occlusion. Circulation 1985;**72**:933-942.

Mason JW, Stinson EB, Winkle RA, et al. Surgery for ventricular tachycardia: efficacy of left ventricular aneurysm resection compared with operation guided by electrical activation mapping. Circulation 1982;**65(6)**:1148-1155.

Mason JW, Hondeghem LM, Katzung BG. Block of inactivated sodium channels and of depolarization-induced automaticity in guinea pig papillary muscle by amiodarone. Circ Res 1984;**55(3)**:277-285.

Matthews SJ, Michelson PA, Cersosimo RJ. Cimetidine-induced sinus bradycardia. Clin Pharm 1982;**1**:556-558.

May GS, Furberg CD, Eberlein KA, Geraci BJ. Secondary prevention after myocardial infarction: a review of short-term acute phase trials. Prog Cardiovasc Dis 1983;**25(4)**:335-359.

Maze M, Smith CM. Identification of receptor mechanism mediating epinephrine-induced arrhythmias during halothane anesthesia in the dog. Anesthesiology 1983;**59(4)**:322-326.

Meade TW, Arie THD, Brewis M, Bond DJ, Morris JN. Recent history of ischaemic heart disease and duodenal ulcer in doctors. Br Med J 1968;**3**:701-704.

Meek WJ. Cardiac automaticity and response to blood pressure raising agents during inhalation anesthesia. Physiol Rev 1941;**21**:324-356.

Menken U, Wiegand V, Bucher P, Meesmann W. Prophylaxis of ventricular fibrillation after acute experimental coronary occlusion

by chronic beta-adrenoceptor blockade with atenolol. Cardiovasc Res 1979;**13(10)**:588-594.

Michelson EL, Spear JF, Moore EN. Electrophysiologic and anatomic correlates of sustained ventricular tachyarrhythmias in a model of chronic myocardial infarction. Am J Cardiol 1980a;**45**:583-590.

Michelson EL, Spear JF, Moore EN. Initiation of sustained ventricular tachyarrhythmias in a model of chronic infarction: importance of the site of stimulation. Am J Cardiol 1980b;**45**:494(abstr).

Michelson EL, Spear JF, Moore EN. Initiation of sustained ventricular tachyarrhythmias in a canine model of chronic myocardial infarction: importance of the site of stimulation. Circulation 1981;**63(4)**: 776-784.

Miletich DJ, Khan A, Albrecht RF, Jozefiak A. Use of heart cell cultures as a tool for the evaluation of halothane arrhythmia. Toxicol Appl Pharmacol 1983;**70**:181-187.

Millar JS, Vaughan Williams EM. Anion antagonism - a fifth class of antiarrhythmic action? Lancet 1981;**i**:1291-1293.

Milner PG, Platia EV, Reid PR, Griffith LSC. Ambulatory electrocardiographic recordings at the time of fatal cardiac arrest. Am J Cardiol 1985;**56**:588-592.

Mines GR. On dynamic equilibrium in the heart. J Physiol 1913;**46**:350-383.

Mirowski M, Mower MM, Reid PR. The automatic implantable defibrillator. In: Harrison DC, ed. Cardiac arrhythmias. Boston: GK Hall,1981;393-398.

Moore EN, Spear JF. Ventricular fibrillation threshold: its physiological and pharmacological importance. Arch Int Med 1975;**135**:446-453.

Moore EN, Spear JF, Horowitz LN, Feldman HS, Moller RA. Electrophysiologic properties of a new antiarrhythmic drug-Tocainide. Am J Cardiol 1978;**41**:703-709.

Moore EN, Spear JF, Michelson EL. Non-canine animal models for evaluating antiarrhythmic efficacy. In: Morganroth J, Moore EN, Dreifus LS, Michelson EL, eds. The evaluation of new antiarrhythmic drugs. The Hague: Martinus Nijhoff,1981;47-53.

Moore EN, Spear JF, Michelson EL, Wetstein L. Evaluation of electrophysiologic factors modifying results of programmed electrical stimulation in animal preparations of myocardial infarction. Circulation 1986;**73(suppl II)**:II.11-II.17.

Morena H, Janse MJ, Fiolet JWT, Krieger WJG, Crijns H, Durrer D. Comparison of the effects of regional ischemia, hypoxia, hyperkalemia, and acidosis on intracellular and extracellular potentials and metabolism in the isolated porcine heart. Circ Res 1980;46(5):636-646.

Morganroth J. Comparative evaluation of antiarrhythmic agents. Drugs 1985;**29(suppl 4)**:14-20.

Moss AJ, DeCamilla J, Davis H. Cardiac death in the first 6 months after myocardial infarction: potential for mortality reduction in the early posthospital period. Am J Cardiol 1977;**39**:816-820.

Moss AJ, Davis HT, DeCamilla J, Bayer LW. Ventricular ectopic beats and their relastion to sudden and nonsudden cardiac death after myocardial infarction. Circulation 1979;**60(5)**:998-1003.

Moss AJ, Davis HT, Conard DL, DeCamilla JJ, Odoroff CL. Digitalis-associated cardiac mortality after myocardial infarction. Circulation 1981;**64(6):**1150-1156.

Mounsey P. Intensive coronary care: Arrhythmias after acute myocardial infarction. Am J Cardiol 1967;**20**:475-483.

Mukharji J, Rude RE, Poole WK, et al. Risk factors for sudden death after myocardial infarction: two-year follow-up. Am J Cardiol 1984;**54**:31-36.

Multicentre International Study. Improvement in prognosis of myocardial infarction by long-term beta-adrenoceptor blockade using practolol. Br Med J 1975;**3**:735-740.

Murray PA, Lavellee M, Vatner SF. Alpha-adrenergic-mediated reduction in coronary blood flow secondary to carotid chemoreceptor reflex activation in conscious dogs. Circ Res 1984;**54**:96-106.

Myerburg RJ, Gelband H, Nilsson K, et al. Long-term electrophysiological abnormalities resulting from experimental myocardial infarction in cats. Circ Res 1977;**41(1)**:73-84.

Myerburg RJ. Sudden death. J Cont Ed Cardiol 1978;**14**:15-29.

Myerburg RJ, Conde S, Sheps DS, et al. Antiarrhythmic drug therapy in survivors of prehospital cardiac arrest: comparison of effects on chronic ventricular arrhythmias and recurrent cardiac arrest. Circulation 1979;**59(5)**:855-863.

Nassar BA, Manku MS, Reed JD, Tynan M, Horrobin DF. Actions of prolactin and frusemide on heart rate and rhythm. Br Med J 1974;**2**:27-29.

Nayler WG, Poole-Wilson PA, Williams A. Hypoxia and calcium. J Mol Cell Cardiol 1979;**11**:683-706.

Nickerson M, Nomaguchi GM. Mechanism of dibenamine protection against cyclopropane-epinephrine cardiac arrhythmias. J Pharmacol Exp Ther 1949;**15**:1-11.

Nickerson M, Brown HO. Protection by dibenamine against "spontaneous" arrhythmias occurring during cyclopropane anesthesia. Anesthesiol 1951;**12**:216-224.

Nikolic G, Bishop RL, Singh JB. Sudden death recorded during holter monitoring. Circulation 1982;**66(1)**:218-225.

Norris RM, Brown MA, Clarke ED, Barnaby PF, Geary GG, Logan RL, Sharpe DN. Prevention of ventricular fibrillation during acute myocardial infarction by intravenous propanolol. Lancet 1984;**ii**:883-886.

Norwegian Multicenter Study Group. Timolol-induced reduction in mortality and reinfarction in patients surviving acute myocardial infarction. New Engl J Med 1981;**304(14)**:801-807.

Nwangwu PU, Holcslaw TL, Stohs SJ. A rapid in vivo technique for preliminary screening of antiarrhythmic agents in mice. Arch Int Pharmacodyn 1977;**229**:219-226.

Okuma K, Sugiyama S, Wada M, et al.Experimental studies on the antiarrhythmic action of a lidocaine analog. Cardiology 1976;**61**:289-297.

Olsson SB, Edvardsson N. Clinical electrophysiologic study of antiarrhythmic properties of flecainide: acute intraventricular delayed conduction and prolonged repolarization in regular paced and premature beats using intracardiac monophasic action potentials with programmed stimulation. Am Heart J 1981;**102(5)**:864-871.

Opie LH, Nathan D, Lubbe WF. Biochemical aspects of arrhythmogenesis and ventricular fibrillation. Am J Cardiol1979;**43**:131-148.

Opie LH, Bruyneel KJ, Lubbe WF. What has the baboon to offer as a model of experimental ischemia? Eur Heart J 1983;**4**:55-60.

Opie LH, Thandroyen FT; Muller CA, Hamm CW. Calcium channel antagonists as antiarrhythmic agents: contrasting properties of verapamil and dilitiazem versus nifedipine. In: Opie LH, ed. Calcium antagonists and cardiovascular disease. New York:Raven Press,1984;303-311.

Oshita S, Sada H, Kojima M, Ban T. Effects of tocainide and lidocaine on the transmembrane action potentials as related to external potassium and calcium concentrations in guinea-pig papillary muscles. Naunyn-Schmiedeberg's Arch Pharmacol 1980;**314**:67-82.

Palileo EV, Welch W, Hoff J, et al. Lack of effectiveness of oral mexiletine in patients with drug-refractory paroxysmal sustained ventricular tachycardia. Am J Cardiol 1980;**50**:1075-1081.

Panidis JP, Morganroth J. Sudden death in hospitalized patients: Cardiac rhythm disturbances detected by ambulatory electro-cardiographic monitoring. J Am Coll Cardiol 1983;**2(5)**:798-805.

Pantridge JF, Adgey AAJ, Geddes JS, Webb SW. The acute coronary attack. Tunbridge Wells: Pitman Medical,1975.

Patterson E, Gibson JK, Lucchesi BR. Electrophysiologic effects of disopyramide phosphate on reentrant ventricular arrhythmias in conscious dogs after myocardial infarction. Am J Cardiol 1980;**46**:792-799.

Patterson E, Gibson JK, Lucchesi BR. Electrophysiologic actions of lidocaine in a canine model of chronic myocardial ischemic damage - arrhythmogenic actions of lidocaine. J Cardiovasc Pharmacol 1982a;**4**:925-934.

Patterson E, Holland K, Eller BT, Lucchesi BR. Ventricular fibrillation resulting from ischemia at a site remote from previous myocardial infarction. Am J Cardiol 1982b;**50**:1414-1423.

Patterson E, Lynch JJ, Lucchesi BR. Antiarrhythmic and antifibrillatory actions of the beta adrenergic receptor antagonist,dl-sotalol. J Pharmacol Exp Ther 1984;**230(2)**:519-526.

Paul O, Schatz M. On Sudden death. Circulation 1971;**43**:7-10.

Penny WJ, Sheridan DJ. The effects of phentolamine on arrhythmias

and cellular electrophysiology during myocardial ischaemia and reperfusion in the guinea-pig. J Physiol 1982;**326**:64p(abstr).

Peters RW, Shafton E, Frank S, Thomas AN, Scheinman MM. Radiofrequency-triggered pacemakers: uses and limitations. Ann Intern Med 1978;**88(1)**:17-22.

Podrid PJ, Schoeneberger A, Lown B, et al. Use of nonsustained ventricular tachycardia as a guide to antiarrhythmic drug therapy in patients with malignant ventricular arrhythmia. Am Heart J 1983;**105(2)**:181-188.

Podrid PJ. The role of antiarrhythmic drugs in prevention of sudden cardiac death. Cardiovasc Clin 1985;**15(3)**:265-286.

Porter WT. On the results of ligation of the coronary arteries. J Physiol 1894;**15**:121-138.

Poser RF, Podrid PJ, Lombardi F, Lown B. Aggravation of arrhythmia induced with antiarrhythmic drugs during electrophysiologic testing. Am Heart J 1985;**110(1)**:9-16.

Pratt CM, Francis MJ, Luck JC, Wyndham CR, Miller RR, Quinones MA. Analysis of ambulatory electrocardiograms in 15 patients during spontaneous ventricular fibrillation with special reference to preceding arrhythmic events. J Am Coll Cardiol 1983;**2(5)**:789-797.

Prevost JL, Battelli F. La mort par les courants electriques: Courants alternatifs a haute tension. J Physiol Path Gen 1899;**1**:427.

Price HL, Ohnishi ST. Effects of anesthetics on the heart. Fed Proc 1980;**39**:1575-1579.

Prinzmetal M, Toyoshima H, Ekmekci A, Mizuno Y, Nagaya T. Nature of ischemic electrocardiographic patterns in mammalian ventricles as determined by intracellular electrographic and metabolic changes. Am J Cardiol 1961;**8**:493-503.

Rabinowitz B, Chuck L, Kligerman M, Parmley WW. Positive inotropic effects of methoxamine: evidence for alpha-adrenergic receptors in ventricular myocardium. Am J Physiol 1975;**229**:582-585.

Rae AP, Greenspan AM, Spielman SR, et al. Antiarrhythmic drug efficacy for ventricular tachyarrhythmias associated with coronary artery disease as assessed by electrophysiologic studies. Am J Cardiol 1985;**55**:1494-1499.

Rai V, Bhatt PS, Singh RH, Vaish SK, Udupa KN. Histamine and histaminase changes in arterial hypertension and myocardial infarction. Indian Heart J 1976;**28(2)**:115-117.

Raine AEG, Vaughan-Williams EM. Possible explanation for protective action of long-term beta-adrenoceptor blockade after myocardial infarction. Br Heart J 1976;**38**:873-882.

Raper C, Wale J. Propanolol, MJ 1999 and Ciba 39089 Ba in ouabain and adrenaline induced cardiac arrhythmias. Eur J Pharmacol 1968;**4**:1-12.

Rashid S, Alps BJ. Futher observations on the experimental antidysrhythmic activity of indoramin hydrochloride. J Pharm Pharmacol 1973;**25**:700-704.

Reding P, Devroede C, Barbier P. Bradycardia after cimetidine. Lancet 1977;**ii**:1227.

Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death: 1. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation 1977;**56**:786-794.

Reynolds AK, Chiz JF, Pasquet AF. Halothane and methoxyflurane - a comparison of their effects on cardiac pacemaker fibers. Anesthesiology 1970;**33**:602-610.

Reynolds EW, Vander Ark CR. Quinidine syncope and the delayed

repolarization syndromes. Mod Concepts Cardiovasc Dis 1976;**45**: 117-122.

Richardson JA. Circulating levels of catecholamines in acute myocardial infarction and angina pectoris. Prog Cardiovasc Dis 1963;**6(1)**:56-62.

Rinkenberger RL, Prystowsky EN, Jackman WM, Naccarelli GV, Heger JJ, Zipes DP. Drug conversion of nonsustained ventricular tachycardia to sustained ventricular tachycardia during serial electrophysiologic studies: Identification of drugs thast exacerbate tachycardia and potential mechanisms. Am Heart J 1982;**103(2)**:177-184.

Ritchie DM, Kelliher GJ, MacMillan A, Fasolak W, Roberts J, Mansukhani S. The cat as a model for myocardial infarction. Cardiovasc Res 1979;**13**:199-206.

Roberts J, Ito R, Reilly J, Cairoli VJ. Influence of reserpine and beta-TM 10 on digitalis-induced ventricular arrhythmias. Circ Res 1963;**13**:149-157.

Roberts WC. Sudden cardiac death: definitions and causes. Am J Cardiol 1986;**57**:1410-1413.

Roden DM, Woosley RL. Class 1 antiarrhythmic agents: quinidine, procainamide and N-acetylprocainamide, disopyramide. Pharmacol Ther 1984;**23**:179-191.

Roizen MF, Moss J, Henry DP, Kopin IJ. Effects of halothane on plasma catecholamines. Anesthesiology 1974;**41**:432-439.

Rosen MR, Gelband H, Merker C, Hoffman BF. Mechanism of digitalis toxicity: Effects of ouabain on phase four of canine Purkinje fiber transmembrane potentials. Circulationm 1973;**47**:681-689.

Rosen MR, Ilvento J, Gelband H, Merker C. Effects of verapamil on the electrophysiologic properties of canine cardiac Purkinje fibers. J

Pharmacol Exp Ther 1974; **189(2)**:414-423.

Rosen MR. Cellular electrophysiology of digitalis toxicity. J Am Coll Cardiol 1985;**5(5)**:22A-34A.

Rosenfeld J, Rosen MR, Hoffman BF. Pharmacologic and behavioural effects on arrhythmias that immediately follow abrupt coronary occlusion: a canine model of sudden coronary death. Am J Cardiol 1978;**41**:1075-1082.

Rossi PR, Yusuf S, Ramsdale D, Furze L, Sleight P. Reduction of ventricular arrhythmias by early intravenous atenolol in suspected acute myocardial infarction. Br Med J 1983;**286**:506-510.

Roy D, Marchlinski FE, Doherty JV, Buxton AE, Waxman HL, Josephson ME. Electrophysiologic testing of survivors of cardiac arrest. In: Josephson ME, ed. Sudden cardiac death. Philadelphia: FA Davis,1985a;171-177.

Roy D, Marchlinski FE, Doherty JV, Buxton AE, Josephson ME. Pharmacologic therapy for survivors of sudden death based on programmed stimulation. In: Josephson ME, ed. Sudden cardiac death. Philadelphia: FA Davis, 1985b;179-185.

Ruskin JN, DiMarco JP, Garan H. Out-of-hospital cardiac arrest. Electrophysiologic observations and selection of long-term antiarrhythmic therapy. New Engl J Med 1980;**303**:607-613.

Ruskin JN, McGovern B, Garan H, DiMarco JP, Kelly E. Antiarrhythmic drugs: A possible cause of out-of-hospital cardiac arrest. New Engl J Med 1983;**309(21)**:1302-1306.

Russell DC, Oliver MF. Ventricular refractoriness during acute myocardial ischaemia and its relationship to ventricular fibrillation. Cardiovasc Res 1978;**12**:221-227.

Russell DC, Smith HJ, Oliver MF. Transmembrane potential changes and ventricular fibrillation during repetitive myocardial ischaemia

in the dog. Br Heart J 1979;**42**:88-96.

Russell DC, Lawrie JS, Riemersma RA, Oliver MF. Mechanisms of phase 1a and 1b early ventricular arrhythmias during acute myocardial ischemia in the dog. Am J Cardiol 1984;**53(2)**:307-313.

Ryden L, Ariniego R, Arnman K, et al. A double-blind trial of metoprolol in acute myocardial infarction: Effects on ventricular tachyarrhythmias. New Engl J Med 1983;**308(11)**:614-618.

Saksena S, Craelius W. The electropharmacology and therapeutic role of mexiletine. Clin Prog Pacing Electrophysiol 1983;**1(2)**:122-141.

Sami M, Chaitman BR, Bourassa MG, Charpin D, Chabot M. Long term follow-up of aneurysmectomy for recurrent ventricular tachycardia or fibrillation. Am Heart J 1978;**96(3)**:303-308.

Schanne OF, Kbaily G. Explanted cardiac cells: a model to study drug actions? Canad J Physiol Pharmacol 1981;**59**:443-467.

Scherlag BJ, Helfant RH, Haft JI, Damoto AN. Electrophysiology underlying ventricular arrhythmias due to coronary ligation. Am J Physiol 1970;**219(6)**:1665-1671.

Scherlag BJ, El-Sherif N, Hope R, Lazzara R. Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction. Circ Res 1974;**35**:372-383.

Schoenfeld MH, Whitford E, McGovern B, Garan H, Ruskin JN. Oral mexiletine in the treatment of refractory ventricular arrhythmias: The role of electrophysiologic techniques. Am Heart J 1984;**5(2)**: 1071-1078.

Schumann HJ, Wagner J, Knorr A, Reidemeister JC, Sadony V, Schramm G.Demonstration in human atrial preparations of a-receptors mediating positive inotropic effects. Naunyn-Schmiedeberg's Arch Pharmacol 1978;**302**:333-336.

Schwartz PJ, Stone HL. Left stellectomy in the prevention of ventricular fibrillation caused by acute myocardial ischemia in conscious dogs with anterior myocardial infarction. Circulation 1980;**62(6)**:1256-1265.

Sedman AJ, Gal J, Mastropaolo W, Johnson P, Maloney JD, Moyer TP. Serum tocainide enantiomer concentrations in human subjects. Br J Clin Pharmacol 1984;**17**:113-115.

Sekiya A, Vaughan-Williams EM. The effects of pronethalol, dichloroisoprenaline and disopyramide on the toxicity to the heart of ouabain and anaesthetics. Br J Pharmacol 1963;**21**:462-472.

Selye H, Bajusz E, Grasso S, Mendell P. Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 1960;**11**:398-407.

Selzer A, Wray HW. Quinidine syncope: paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias. Circulation 1964;**30**:17-26.

Shah RR. Symptomatic bradycardia in association with H2-receptor antagonists. Lancet 1982;**ii**:1108.

Shanks RG, Dunlop D. Effect of propanolol on arrhythmias following coronary artery occlusion in dogs. Cardiovasc Res 1967;**1**:34-41.

Shanks RG. The properties of beta-adrenoceptor antagonists. Postgrad Med J 1976;**52(suppl 4)**:14-20.

Sharma AD, Corr PB. Adrenergic factors in arrhythmogenesis in the ischemic and reperfused myocardium. Eur Heart J 1983; **4(suppl D)**:79-90.

Sharma AD, Saffitz JE, Lee BJ, Sobel BE, Corr PB. Alpha adrenergic mediated accumulation of calcium in reperfused myocardium. J Clin Invest 1983;**72(3)**:802-818.

Shaw RG, Mashford ML, Desmond PV. Cardiac arrest after intravenouys administration of cimetidine. Med J Aust 1980;**2**:629-630.

Sheridan DJ, Penkoske PA, Sobel BE, Corr PB. Alpha adrenergic contributions to dysrhythmia duiring myocardial ischemia and reperfusion in cats. J Clin Invest 1980;**65**:161-171.

Sheridan DJ, Culling W. Electrophysiological effects of α-adrenoceptor stimulation in normal and ischemic myocardium. J Cardiovasc Pharmacol 1985;**7(suppl 5)**:S55-S60.

Shibuya T, Hashimoto K, Imai S. Effective plasma concentrations of antiarrhythmic drugs against halothane-adrenaline arrhythmia in dogs. J Cardiovasc Pharmacol 1983;**5(4)**:538-545.

Simpson PJ, Mickelson J, Fantone JC, Gallagher KP, Lucchesi BR. Iloprost inhibits neutrophil function in vitro and in vivo and limits experimental infarct size in canine heart. Circ Res 1987;**60(5)**: 666-673.

Simson MB, Spear J, Moore EN. The relationship between atrioventricular nodal refractoriness and the functional refractory period in the dog. Circ Res 1979;**44(1)**:121-126.

Singh BN, Vaughan Williams EM. The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. Br J Pharmacol 1970;**39**:657-667.

Singh BN, Vaughan Williams EM. A fourth class of anti-dysrhythmic action? Effect of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. Cardiovasc Res 1972a;**6**:109-119.

Singh BN, Vaughan Williams EM. Investigations of the mode of action of a new antidysrhythmic drug, Ko 1173. Br J Pharmacol 1972b;**44**:1-9.

Singh BH, Nadamanee K. The electrophysiological classification of antiarhythmic drugs. In: Coltart DG, Jewitt DE, eds. Recent developments in cardiovascular drugs. London: Churchill-Livingstone, 1982;59-77.

Smith FM. The ligation of coronary arteries with electrocardiographic study. Arch Int Med 1918;**7**:8-27.

Somani P, Lum KB. The antiarrhythmic actions of beta adrenergic blocking agents. J Pharmacol Exp Ther 1965;**147(2)**:194-204.

Somberg J, Tepper D, Wynn J. Prolonged repolarization: A historical perspective. Am Heart J 1985;**109**:395-398.

Sowton E, Leatham A, Carson P. The suppression of arrhythmias by artificial pacemaking. Lancet 1964; **ii**:1098-1100.

Spear JF. Relationship between the scalar electrocardiogram and cellular electrophysiology of the rat heart. In: Budden R, Detweiler DK, Zbinden G, eds. International workshop on the rat electrocardiogram in acute and chronic pharmacology and toxicology. Oxford: Pergamon Press,1981;29-41.

Sperelakis N. Properties of calcium-dependent slow action potentials: their possible role in arrhythmias. In: Opie LH, ed. Calcium antagonists and cardiovascular disease. New York: Raven Press,1984;277-291.

Spiekermann RE, Brandenberg JT, Achor RWP, Edwards JE. The spectrum of coronary heart disease in a community of 30,000. Circulation 1962;**25**:57-65.

Standaert FG, Levitt B, Robert J, Raines A. Antagonism of ventricular arrhythmias induced by digitalis - a neural phenomenon. Eur J Pharmacol 1969;**6**:209-216.

Stephenson SE, Cole RK, Parrish TF, et al. Ventricular fibrillation during and after coronary occlusion: incidence and protection

afforded by various drugs. Am J Cardiol 1960;**5**:77-87.

Stevenson WG, Brugada P, Waldecker B, Zehender M, Wellens HJJ. Clinical, angiographic and electrophysiologic findings in patients with aborted sudden death as compared with patients with sustained ventricular tachycardia after myocardial infarction. Circulation 1985;**71(6)**:1146-1152.

Stimmesse B, Daoudal P, Neidhardt A. Bradycardie au cours d'un traitement par cimetidine. Nouvelle Presse Med 1978;**7**:4233.

Strauss HC, Bigger JT, Hoffman BF. Electrophysiological and beta-receptor blocking effects of MJ 1999 on dog and rabbit cardiac tissue. Circ Res 1970;**26(6)**:661-678.

Struthers AD, Reid JL, McLean K, Rodger JC. Adrenaline, hypokalaemia and cardiac arrhythmias: Effects of beta adrenoceptor antagonists. Clin Sci 1981;**62**:1p(abstr).

Swerdlow CD, Winkle RA, Mason JW. Determinants of survival in patients with ventricular tachyarrhythmias. N Engl J Med 1983; **308(24)**:1436-1442.

Thandroyen FT, Worthington MG, Higgingson LM, Opie LH. The effects of alpha- and beta- adrenoceptor antagonist agents on reperfusion ventricular fibrillation and metabolic status in the isolated perfused rat heart. J Am Coll Cardiol 1983;**1(4)**:1056-1066.

The Coronary Drug Project Research Group. Prognostic importance of prmature beats following myocardial infarction. JAMA 1973;**223(10)**: 1116-1124.

The MIAMI Trial Research group. Metoprolol in acute myocardial infarction (MIAMI): A Randomised placebo-controlled international trial. Eur Heart J 1985;**6**:199-226.

The Multicenter Postinfarction Research Group.Risk stratification and survival after myocardial infarction. New Engl J Med

1983;**309(6)**: 331-336.

Theroux P, Waters DD, Halphen C, Debaisieux J-C, Mizgala HF. Prognostic value of exercise testing soon after myocardial infarction. New Engl J Med 1979;**301(7)**:341-345.

Thomas M, Jewitt DE, Shillingford JP. Analysis of 150 patients with acute myocardial infarction admitted to an intensive care and study unit. Br Med J 1968;**1**:787-790.

Thomas JX, Jones CE, Randall WC. Neural modulation of coronary blood flow. In: Randall WC, ed. Nervous control of cardiovascular function. New York: Oxford University Press,1984;178-198.

Thompson PL, Lown B. Sequential R/T pacing to expose electrical instability in the ischemic ventricle. Clin Res 1972;**20**:401(abstr).

Toda N. Mechanism of histamine actions in human coronary arteries. Circ Res 1987;**61(2)**:280-286.

Torres V, Flowers D, Somberg JC. The arrhythmogenicity of antiarrhythmic agents. Am Heart J 1985;**109(5)**:1090-1097.

Touboul P, Atallah G, Gressard A, et al. Electrophysiological study on mexiletine in man with reference to dose-response relation. Arch Mal Coeur 1978;**71(12)**:1429-1437.

Tsien RW, Giles W, Greengard P. Cyclic AMP mediates the effects of adrenaline on cardiac Purkinje tissues. Nature (New Biol) 1972;**240**:181-183.

Vassalle M, Greenspan K, Hoffman BF. An analysis of arrhythmias induced by ouabain in intact dogs. Circ Res 1963;**13**:132-148.

Vassalle M. Electrogenic suppression of automaticity in sheep and dog Purkinje fibers. Circ Res 1970;**27**:361-377.

Vassalle M. Automaticity and automatic rhythms. Am J Cardiol

1971;**28**:245-252.

Vaughan Williams EM, Sekiya A. Prevention of arrhythmias due to cardiac glycosides by block of beta sympathetic receptors. Lancet 1963;**i**:420-421.

Vaughan Williams EM. Classification of antiarrhythmic drugs. In: Sando E, Flensted-Jensen EG, Oleson KH, eds. Symposium on cardiac arrhythmia. Sodertalje: AB Astra, 1970.

Vaughan Williams EM. Classification of antidysrhythmic drugs. Pharmacol Ther 1975;**1(1)**:115-138.

Vaughan Williams EM. A classification of antiarrhythmic action reassessed after a decade of new drugs. J Clin Pharmacol 1984;**24**:129-147.

Vaughan Williams EM. Cardiac electrophysiological effects of selective adrenoceptor stimulation and their possible roles in arrhythmias. J Cardiovasc Pharmacol 1985;**7(suppl 5)**:S61-S64.

Velebit V, Podrid P, Lown B, Cohen BH, Graboys TB. Aggravation and provocation of ventricular arrhythmias by antiarrhythmic drugs. Circulation 1982;**65(5)**:886-894.

Verdouw PD, Remme WJ, Hugenholtz PG. Cardiovascular and antiarrhythmic effects of aprindine (AC 1802) during partial occlusion of a coronary artery in the pig. Cardiovasc Res 1977; **11**:317-323.

Verdouw PD, Wolffenbuttel BHR, Van der Giessen WJ. Domestic pigs in the study of myocardial ischemia. Eur Heart J 1983;**4**:61-67.

Vick RL. Effects of altered heart rate on chloroform-epinephrine cardiac arrhythmias. Circ Res 1966;**18**:316-322.

Vismara LA, Amsterdam EA, Mason DT. Relation of ventricular arrhythmias in the late hospital phase of acute myocardial

infarction to sudden death after hospital discharge. Am J Med 1975;**59(1)**:6-12.

Vleugels A, Carmeliet EE. Hypoxia increases potassium efflux from mammalian myocardium. Experientia 1976;**32**:483-484.

Volosin K, Greenberg RM, Greenspon AJ. Tocainide associated agranulocytosis. Am Heart J 1985;**109**:1392-1393.

Vugman I, Rocha e Silva M. Biological determination of histamine in living tissues and body fluids. In: Rocha e Silva M, ed. Handbook of experimental pharmacology. New York: Springer-Verlag,1966;81-136.

Wagner J, Brodde OE. On the presence and distribution of alpha-adrenoceptors in the heart of various mammalian species. Naunyn-Schmiedeberg's Arch Pharmacol 1978;**302**:239-254.

Waldo AL, Kaiser GA. A study of ventricular arrhythmias associated with acute myocardial infarction in the canine heart. Circulation 1973;**47**:1222-1228.

Walker MJA, Curtis MJ, Hearse DJ, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. Cardiovasc Res (submitted).

Wallace AG.Electrical activity of the heart. In: Hurst JW, ed. The heart. New York: McGraw-Hill, 1982; 115-127.

Ward OC. A new familial cardiac syndrome in children. J Irish Med Assoc 1964;**54(322)**:103-106.

Waspe LE, Waxman HL, Buxton AE, Josephson ME. Mexiletine for control of drug-resistant ventricular tachycardia: clinical and electro- physiologic results in 44 patients. Am J Cardiol 1983;**51**:1175-1181.

Waspe LE, Seinfeld D, Ferrick A, Kim SG, Matos JA, Fisher JD.

Prediction of sudden death and spontaneous ventricular tachycardia in survivors of complicated myocardial infarction: value of the response to programmed stimulation using a maximum of three ventricular extrastimuli. J Am Coll Cardiol 1985;**5(6)**:292-301.

Watanabe AM, Hathaway DR, Besch HR, Farmer BF, Harris RA. Alpha-adrenergic reduction of cyclic adenosine monophosphate concentrations in rat myocardium. Circ Res 1977;**40(6)**:596-602.

Watson AJS, Watson R, Keogh JAB. Cimetidine induced arrhythmias. Ir J Med Sci 1982;**51(11)**:348-350.

Weidmann S. Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. J Physiol 1955;**129**: 568-582.

Weidman S. Shortening of the action potential due to brief injections of KCl following the onset of activity. J Physiol 1956;**132**:156-163.

Wellens HJJ, Schuilenberg RM, Durrer D. Electrical stimulation of the heart in patients with ventricular tachycardia. Circulation 1972; **46(2)**:216-226.

Wellens HJJ, Lie KI, Durrer D. Further observation on ventricular tachycardia as studied by electrical stimulation of the heart: chronic recurrent ventricular tachycardia and ventricular tachycardia during acute myocardial infarction. Circulation 1974;**49(4)**:647-653.

Wellens HJJ. Electrical stimulation of the heart in the study and treatment of tachycardias. Baltimore: University Park Press,1977.

Whitsitt LS, Lucchesi BR. The cardiac bete-adrenergic receptor blocking actions of propanolol and its stereoisomers. Life Sci 1967;**6**:939-950.

Wilber DJ, Lynch JJ, Montgomery DG, Lucchesi BR. Postinfarction sudden death:significance of inducible ventricular tachycardia and

infarct size in a conscious canine model. Am Heart J 1985;**109(1)**: 8-18.

Wilber DJ, Lynch JJ, Montgomery DG, Lucchesi BR. Alpha-adrenergic influences in canine ischemic sudden death: effects of alpha 1-adrenoceptor blockade with prazosin. J Cardiovasc Pharmacol 1987; **10**:96-106.

Wilkerson RD, Downey JM. Ventricular arrhythmias produced by coronary artery occlusion in closed-chest dogs. J Pharm Meth 1978;**1**:39-44.

Williams DO, Scherlag BJ, Hope RR, El-Sherif N, Lazzara R. The pathophysiology of malignant ventricular arrhythmias during acute myocardial ischemia. Circulation 1974;**50**:1163-1172.

Winkle RA, Mason JW, Griffin JC, Ross D. Malignant ventricular tachyarrhythmias associated with the use of encainide. Am Heart J 1981;**102(5)**:857-864.

Winslow E. Methods for the detection and assessment of antiarrhythmic activity. Pharmacol Ther 1984;**24(3)**:401-433.

Wit AL, Cranefield PF. Effect of verapamil on the sinoatrial and atrioventricular nodes of the rabbit and the mechanism by which it arrests reentrant atrioventricular nodal tachycardia. Circ Res 1974;**35**:413-425.

Wit AL, Rosen MR, Hoffman BF. Electrophysiology and pharmacology of cardiac arrhythmias. II. Relationship of normal and abnormal electrical activity of cardiac fibers to the genesis of arrhythmias. A. Automaticity. Am Heart J 1974a;**88**:515-524.

Wit AL, Rosen MR, Hoffman BF. Electrophysiology and pharmacology of cardiac arrhythmias. II. Relationship of normal and abnormal electrical activity of cardiac fibers to the genesis of arrhythmias. B. Reentry. Section 1. Am Heart J 1974b;**88**:664-670.

Wit AL, Rosen MR, Hoffman BF. Electrophysiology and pharmacology of cardiac arrhythmias. II. Relationship of normal and abnormal electrical activity of cardiac fibers to the genesis of arrhythmias. B Reentry. Section II. Am Heart J 1974c;**88**:798-806.

Witkowski FX, Corr PB. Mechanisms responsible for arrhythmias associated with reperfusion of ischemic myocardium. Ann NY Acad Sci 1984;**427**:187-198.

Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: an overview of the randomised trials. Prog Cardiovasc Dis 1985;**27(5)**: 335-371.

Zelis RF, Wenger NK. Prevention of coronary atherosclerosis. In: Hurst JW, ed. The heart. New York: McGraw-Hill, 1982; 959-976.

Zimmerman JM, Bigger JT, Coromilas J. Flecainide acetate is arrhythmogenic in a canine model of sustained ventricular tachycardia. J Am Coll Cardiol 1985;**5**:391(abstr).

Zipes DP, Fischer JC. Effects of agents which inhibit the slow channel on sinus node automaticity and atrioventricular conduction in the dog. Circ Res 1974;**34**:184-192.

Zipes DP, Troup PJ. New antiarrhythmic agents. Am J Cardiol 1978;**41**:1005-1024.

Zoll PM, Linenthal AJ, Gibson W, Paul MH, Norman LR. Termination of ventricular fibrillation in man by externally applied countershock. New Engl J Med 1956;**254**:727-732.

Addendum

Caillard CG, Louis JC. Assessment of antiarrhythmic drugs in experimental pharmacology. Meth Find Exp Clin Pharmacol 1980;**2(5)**:223-252.